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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/200,791
Filing Date	November 30, 1998
First Named Inventor	Thomas M. BEHR et al.
Group Art Unit	1642
Examiner Name	FETTEROLF, Brandon J.
Attorney Docket Number	41058-0016 US1
Total Number of Pages in This Submission	

ENCLOSURES *(check all that apply)*

- [x] Fee Transmittal Form
  - [x] Fee Attached
- [ ] Amendment / Response
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [x] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) *(please identify below)*:
  Return Receipt Postcard;
  6 references cited in Appendix IX

Remarks	**CUSTOMER NO. 26633**

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm *or* Individual name	Patricia D. Granados, Reg. No. 33,683, HELLER EHRMAN LLP
Signature	Patricia D. Granados
Date	November 7, 2005

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on this date:

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Signature		Date	

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COMBINED FEE TRANSMITTAL for FY 2005

Effective 12/08/2004. Patent fees are subject to annual revision.

PTO/SB/17 (12-04) (Revised) (For payment of 37 CFR 1.17 fees including (f), (g), (h), & (i))

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 250.00

Complete if Known	
Application Number	09/200,791
Filing Date	November 30, 1998
First Named Inventor	Thomas BEHR et al.
Examiner Name	FETTEROLF, Brandon J.
Art Unit	1642
Attorney Docket No.	41058-0016US1

NOV 07 2005 PATENT & TRADEMARK OFFICE IAP45

METHOD OF PAYMENT (check one)

☒ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None
☐ Deposit Account

Deposit Account Number: 08-1641

Deposit Account Name: Heller Ehrman LLP

The Commissioner is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below
☒ Credit any overpayments and charge any deficiencies
☐ Charge any additional fee(s) during the pendency of this application
☐ Charge fee(s) indicated below, **except for the filing fee** to the deposit account

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
Application Type	**Entity Fee ($)**	**Small Entity Fee ($)**	**Entity Fee ($)**	**Small Entity Fee ($)**	**Entity Fee ($)**	**Small Entity Fee ($)**	**Fees Paid ($)**
Utility	300	150	500	250	200	100	
Design	200	100	100	50	135	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

SUBTOTAL (1) $

2. EXTRA CLAIM FEES

Entity Fee ($)	Small Entity Fee ($)	Fee Description
50	25	Each claim in excess of 20 or, for Reissues, each claim in excess of 20 and more than in the original patent
200	100	Each Independent claim in excess of 3 or, for Reissues, each independent claim more than in the original patent
360	180	Multiple dependent claim, if not already paid

			Extra Claims		Fee from above		Fee Paid
Total Claims	37	-40** =		x		=	0
Independent Claims	3	-3** =	0	x	100	=	0
Multiple Dependent					0	=	0

***or number previously paid, if greater; For Reissues see below*

SUBTOTAL (2) 0

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof (round up to the a whole number). See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s)

Total Sheets		Extra Sheets		Number of each additional 50	Fee ($)		Small Entity Fee ($)
	-100 =		/50 =		x 250	OR	x 125

SUBTOTAL (3) $

FEE CALCULATION (continued)

4. PETITION FEES UNDER 37 CFR 1.17 (f) — Fee Paid:
Fee Code: 1462 **Fee $ 400** For petitions filed under: § 1.53(e); § 1.57(a); § 1.182; § 1.183; § 1.378(e); § 1.741(b)

5. PETITION FEES UNDER 37 CFR 1.17 (g) — Fee Paid:
Fee Code: 1463 **Fee $ 200** For petitions filed under: § 1.12; § 1.14; § 1.47; § 1.59; § 1.103(a); § 1.136(b); § 1.295; § 1.296; § 1.377; § 1.550(c); § 1.956; § 5.12; § 5.15; § 5.25

6. PETITION FEES UNDER 37 CFR 1.17 (h) — Fee Paid:
Fee Code: 1464 **Fee $ 130** For petitions filed under: § 1.19(g); § 1.84; § 1.91; § 1.102(d); § 1.138(c); § 1.313; § 1.314

7. PROCESSING FEES UNDER 37 CFR 1.17 (i) — Fee Paid:
Fee Code: 1808 (1803 for **§ 1.221**) **Fee $ 130** For petitions filed under: § 1.28(c)(3); § 1.41; § 1.48; § 1.52(d); § 1.53(b)(3); § 1.55; § 1.99(e); § 1.103(b); § 1.103(c); § 1.103(d); § 1.217; § 1.221; § 1.291(c)(5); § 1.497(d); § 3.81

8. OTHER FEES

Entity Fee ($)	Entity Fee ($)	Fee Description	Fee Paid
130	65	Surcharge - late filing fee or oath	
50	25	Surcharge - late provisional filing fee or cover sheet	
130	130	Non-English specification	
2,520	2,520	For filing a request for *ex parte* reexamination	
920*	920*	Requesting publication of SIR prior to Examiner action	
1,840*	1,840*	Requesting publication of SIR after Examiner action	
120	60	Extension for reply within first month	
450	225	Extension for reply within second month	
1,020	510	Extension for reply within third month	
1,590	795	Extension for reply within fourth month	
2,160	1,080	Extension for reply within fifth month	
500	250	Filing a brief in support of an appeal	250.00
790	395	Filing a submission after final rejection (37 CFR 1.129(a))	
1,510	1,510	Petition to institute a public use proceeding	
500	250	Petition to revive - unavoidably abandoned application	
1,500	750	Petition to revive - unintentionally abandoned application	
50	50	Processing fee for provisional appls (37 CFR 1.17(q))	
180	180	Submission of Information Disclosure Statement	
1,000	500	Request for oral hearing	
790	395	For each additional invention to be examined (37 CFR 1.129(b))	
790	395	Request for Continued Examination (RCE)	
900	900	Request for expedited examination of a design application	

Other fee (specify) 1401 Notice of Appeal

SUBTOTAL (4+5+6+7+8) $250.00

* Reduced by Basic Filing Fee Paid

SUBMITTED BY

Name *(Print/Type)*	Patricia D. Granados	Registration No. *(Attorney/Agent)*	33,683	Complete *(if applicable)* *Telephone* 202-912-2142
Signature	Patricia D. Granados	*Date*	Nov 7, 2005	Customer No. 26633

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Attorney Docket No.: 41058-0016 US1

In re patent application of: Thomas M. Behr

Confirmation No.: 9799

Application No.: 09/200,791

Art Unit: 1642

Filing Date: November 30, 1998

Examiner: Brandon J. Fetterolf

For: Methods for Reduced Renal Uptake of Protein Conjugates

APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Heller Ehrman LLP

11/08/2005 SZEWDIE1 00000095 09200791
01 FC:2402 250.00 OP

APPEAL BRIEF

I. REAL PARTY IN INTEREST

Immunomedics, Inc. of 300 American Road, Morris Plains, NJ 07950 as assignee, owns the entire right, title and interest in the captioned application and, therefore, is the real party in interest.

II. RELATED APPEALS AND INTERFERENCES

Appellants are aware of no other current appeals, interferences or judicial proceedings which may be related to, directly affect or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1-9, 11-21, 23-29 and 31-41 are pending, stand finally rejected and are under appeal. A copy of the claims on appeal are appended to this brief.

Claims 10, 22, 30 and 42 and 43 have been canceled without prejudice or disclaimer.

IV. STATUS OF AMENDMENTS

All amendments of record have been entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The invention relates to a method of reducing kidney retention of a protein conjugate in a patient, comprising administering to the patient one or more compounds selected from the group consisting of D-lysine, poly-lysine having a molecular weight in the range 1-60 kD, pharmaceutically acceptable salts thereof and carboxyl derivatives

thereof, wherein said protein conjugate has a molecular weight that is not greater than about 60 kD. Additionally, the pharmaceutically acceptable salt and carboxyl derivative of poly-lysine has a molecular weight in the range 1-60 kD. In one embodiment, both D-lysine and poly-lysine are administered to a patient. The poly-lysine may have a molecular weight of 15-30 kD and may be poly-D- or poly-L-lysine.[1]

The compound or compounds reduce kidney retention of the conjugates.[2]

In one embodiment, the protein conjugate may be peptide conjugates, polypeptide conjugates, glycoprotein conjugates, lipoprotein conjugates, antibody conjugates, or antibody fragment conjugates. Such conjugate may be a radiolabeled conjugate, e.g., an imaging isotope, therapeutic isotope.[3]

In another embodiment, the protein conjugate is a radiolabeled hapten conjugate or hapten conjugated to a cytotoxic agent. In another embodiment, the protein conjugate comprises a cytotoxic agent.[4]

In another embodiment, the invention further relates to a method of reducing kidney retention of a protein conjugate in a patient undergoing treatment with a targeting protein conjugate comprising administering to the patient, one or more of a compound that is D-lysine or poly-lysine having a molecular weight in the range 1-60 kD, a pharmaceutically acceptable salt thereof and carboxyl derivatives thereof. The protein conjugate has a molecular weight that is not greater than about 60 kD. The pharmaceutically acceptable salt and carboxyl derivative of poly-lysine has a molecular

[1] Original claim 1; specification at page 2, lines 13-19, at page 3, lines 13-23.

[2] Specification at page 4, lines 24-28.

[3] Specification at page 11, line 31-page 12, line 8.

[4] Specification at page 7, lines 16-28, page 9, lines 18-33.

weight in the range 1-60 kD. The poly-lysine may have a molecular weight of 15-30 kD and may be poly-D- or poly-L-lysine.[5]

The compound or compounds reduce kidney retention of the conjugates.[6]

The protein conjugate is a peptide conjugate, polypeptide conjugate, glycoprotein conjugate, lipoprotein conjugate, antibody conjugate or antibody fragment conjugate. the targeting protein conjugate may comprise a ribonucleic acid binding protein, which may be a ribonuclease, particularly Onconase®. The protein conjugate may be a radiolabeled conjugate, which may be an imaging isotope or a therapeutic isotope.[7]

In another embodiment, the protein conjugate may be a radiolabeled hapten conjugate or a hapten conjugated to a cytotoxic agent. In another, the protein conjugate comprises a cytotoxic agent.[8]

In one embodiment, the compound in the above methods is parenterally administered to the patient in a physiologically acceptable aqueous solution, which may be by continuous infusion or by means of at least one injection of a bolus of the solution. If the administration is by means of at least one injection of a bolus of the solution, it may be followed by oral administration in a physiologically acceptable carrier. In another embodiment, the compound of the invention is orally administered to the patient in a physiologically acceptable carrier.[9]

The invention further relates to a cancer therapeutic or diagnostic method comprising administering to a patient in need thereof a protein conjugate comprising a

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[5] Original claim 1; specification at page 2, lines 13-19; at page 3, lines 13-23.

[6] Specification at page 4, lines 24-28.

[7] Specification at page 8, lines 5-10; at page 7, lines 5-9; at page 10, lines 3-22.

[8] Specification at page 7, lines 16-28; at page 9, lines 18-33.

[9] Specification at page 14, lines 26-34.

cytotoxic agent or an imaging isotope, wherein the protein conjugate has a molecular weight that is not greater than about 60 kD, the improvement comprising additionally administering to the patient one or more compounds that are D-lysine or poly-lysine having a molecular weight in the range 1-60 kD, pharmaceutically acceptable salts thereof and carboxyl derivatives thereof, to reduce kidney retention of said cytotoxic agent or imaging isotope.[10]

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claims 1-8, 11-19, 23-28, 31-39 and 41 are rejected under 35 USC § 102(b) over Behr *et al., Cancer Research* 55: 3825-3834 (September 1,1995) ("Behr").

2. Claims 1-9, 11-19, 23-28, 31-39 and 41 are rejected under 35 USC § 103(a) over Behr, in view of Grey *et al.*, U.S. Patent No. 5,380,513 and Raines *et al.,* U.S. Patent No. 5,840,296.

VII. ARGUMENT

A. Claims 1-8, 11-19, 23-28, 31-39 and 41 are not anticipated by Behr

Behr does not anticipate the subject matter of Claims 1-8, 11-19, 23-28, 31-39 and 41 because Behr is not prior art against these claims. This is so because Behr published on September 1, 1995 and the rejected claims are entitled to the benefit of the filing date of USSN 08/407,899 ("the '899 application"), **March 21, 1995**, which is prior to September 1, 1995. Appellants' reliance upon this 1995 priority date is the primary point of dispute between Appellants and the Examiner. Appellants argue that all the claims are entitled to the 1995 priority date; the Examiner argues that they are not. If Appellants prevail on this point, all rejections are moot.

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[10] Specification at page 10, line 29 to page 11, line 30.

The Examiner does not articulate a specific statutory or legal basis for his denial of priority; rather, the Examiner simply states that "the species of antibodies does not support the genus of just any protein conjugate."[11] Appellants argue that the Examiner is wrong as a matter of law, if the Examiner believes that a species can never support a genus, and is wrong as a matter of fact with regard to this case in particular.

The present application is a continuation-in-part ("CIP") of the '899 application. The basic rule is that in order for a claim in a CIP to be entitled to the filing date of an earlier application, the earlier application must comply with the requirements of 35 USC § 112, first paragraph, with regard to the later claimed subject matter. Determinations of priority are mostly concerned with the written description requirement of 35 USC § 112, first paragraph. In order to meet the written description requirement, the applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was **in possession of the invention.** *Vas-Cath Inc. v. Mahurkar* 935 F.2d 1555, 19 USPQ 2d 1111 (Fed. Cir. 1991) (emphasis added). Thus, the adequacy of the description is judged from the viewpoint of one of ordinary skill in the art of the invention and involves questions of fact.

In a recent case, *Pandrol USA, LP et al. v. Airboss Railway Products, Inc. et al.* (Slip. Op. 04-1069) (September 19, 2005), the U.S. Court of Appeals for the Federal Circuit applied the law of *Vas-Cath* in considering whether a claim reciting "adhering material" was supported by an original description in the specification of two examples of materials that functioned as adhesive materials: an HDPE closed cell foam pad and an epoxy. The court concluded that because such materials were, in fact, adhering materials, the written description requirement for claimed "adhering materials" was met. In *Pandrol*, the disclosure of two species was sufficient to support the recitation of a genus. Thus, as a matter of law, a species may be sufficient to support a genus.

[11] Office Action of October 21, 2004, page 3, paragraph 7.

Appellants understand that each case is different, as each set of facts is different. However, here, the facts support a finding that Appellants' original 1995 disclosure of antibody conjugates supports the present claims reciting protein conjugates. Just as a foam pad was an adhesive material in *Pandrol*, here an antibody conjugate is a type of protein conjugate. Moreover, just as the foam pad in *Pandrol functioned as* an adhesive material, the disclosed antibody *functions as* a protein conjugate. In fact, the problem solved in the '899 application using antibodies is the same problem solved by protein conjugates in the present application.

Specifically, the '899 application, which matured into U.S. Patent No. 5,843,894 ("the 894 patent") discloses a solution to the problem involving the renal uptake of molecules that are smaller than 60 kD. (The '894 patent at column 1, lines 33-36) This is a particular problem with immunotherapy and immunodiagnostics where such molecules are labeled with radioisotopes. The '894 patent describes how molecules smaller than 60 kD are filtered by the glomeruli and subsequently reabsorbed in the renal tubule for subsequent lysosomal degradation. When such molecules are radiolabeled, the radiometals stay in the kidney binding to intracellular proteins with high affinity for metal ions. (Column 1, lines 33-38) The retention of radiometals in the kidney leads to nephritic syndrome and renal insufficiency. The '894 patent describes how others have recognized this problem and offered other solutions, namely treatment with basic L-amino acids to reduce the uptake of radiolabeled peptides and antibody fragments. (Column 1, lines 39-50)

Although the above discussion is mostly in the "Background of the Invention" part of the specification, this discussion is highly relevant to the claimed invention because it defines the problem addressed by the claimed invention. More specifically, it defines the universe of proteins that creates the problem solved by the invention. What the proteins in that universe have in common is a certain size which causes them to be retained in the kidney. Although the precise mechanism by which antibody

fragments find their way into and are retained by the kidney is not explained in the specification, it is clear that antibody fragments conjugated to radiometals and cytotoxic agents do find their way into the kidney where the consequent retention of radiometals and cytotoxic agents can cause renal nephrotoxicity. The invention provides methods to reduce or eliminate this problem. The method by which this problem is solved in the '894 patent is the same method claimed in the present application, *i.e.* the administration to a patient of one or more compounds selected from the group consisting of D-lysine, poly-lysine having a molecular weight in the range 1-60 kD, pharmaceutically acceptable salts thereof and carboxyl derivatives thereof.

Appellants further take issue with the Examiner's statement that "the species of antibodies does not support the genus *of just any protein conjugate*." *Supra.* (Emphasis added) Claims 1 and 38 are not directed to *just any protein conjugate*. These claims recite a protein conjugate having a specific molecular weight: **a molecular weight not greater than about 60 kD**. This is the same size molecule described in the '894 patent as being filterable by the glomeruli and thereby retained in the kidney. Thus, claims 1 and 38 recite the type of protein conjugate addressed by the invention claimed in the '894 patent.

Present claim 18 also recites a size limitation of the protein conjugate and further describes the conjugate as being a "targeting protein conjugate." It is clear that the '894 patent describes targeting protein conjugates, *i.e.* antibody fragments conjugated with radioisotopes or cytotoxic agents. Applicants draw the Examiner's attention to these claim recitations to emphasize their connection to the basic invention disclosed in the '894 patent. The invention described and claimed in the '894 patent is a protein conjugate that is capable of being retained by the kidney. Such proteins are of a size that permits filtration by the glomeruli. In the '894 patent, such proteins are antibody fragments; in the present application such proteins could be antibody fragments or any other protein conjugate of a specific size that is retained in the kidney.

One of ordinary skill in the art of the invention, knowing that antibody conjugates are protein conjugates and that the method described in the '899 application is the same method, for the same purpose as the method described in the present application, would understand that Appellants were in possession of the claimed invention at the time of filing the '899 application.

With regard to claims 2 and 19, which recite specific types of protein conjugates, Appellants argue that the above analysis is applicable. That is, Appellants have included peptide conjugates, polypeptide conjugates, glycoprotein conjugates lipoprotein conjugates, antibody conjugates and antibody fragment conjugates in the same Markush claim because each member is type of protein conjugate. As such, all are entitled to the March 21, 1995 filing date.

Because Behr is not prior art against any of the claims for the above reasons, Appellants respectfully request the Board to reverse the Examiner's rejection under 35 USC § 102(b) of claims 1-8, 11-19, 23-28, 31-39 and 41.

B. Claims 1-9, 11-19, 23-28, 31-39 and 41 are not obvious under 35 USC § 103(a) over Behr, in view of Grey *et al.*, U.S. Patent No. 5,380,513 and Raines *et al., U.*S. Patent No. 5,840,296.

The Examiner's obviousness rejection is defective because Behr is not a proper prior art reference. Appellants reiterate the above arguments with regard to the Examiner's rejection under 35 USC § 102 (b) and add that the secondary references cannot sustain an obviousness rejection without Behr. Thus, the Examiner's obviousness rejection of claims 1-9, 11-19, 23-28, 31-39 and 41 should be reversed.

* * *

In view of the above arguments and evidence of record, Appellants respectfully request the Board to reverse the Examiner's rejection of claims 1-9, 11-19, 23-28, 31-39 and 41.

Respectfully submitted,

November 7, 2005

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VIII. CLAIMS APPENDIX

1. A method of reducing kidney retention of a protein conjugate in a patient, comprising administering to said patient one or more compounds selected from the group consisting of D-lysine, poly-lysine having a molecular weight in the range 1-60 kD, pharmaceutically acceptable salts thereof and carboxyl derivatives thereof, wherein said protein conjugate has a molecular weight that is not greater than about 60 kD

wherein the pharmaceutically acceptable salt and carboxyl derivative of poly-lysine has a molecular weight in the range 1-60 kD,

whereby said compound or compounds reduce kidney retention of said conjugates.

2. A method according to claim 1, wherein said protein conjugate is selected from the group consisting of peptide conjugates, polypeptide conjugates, glycoprotein conjugates, lipoprotein conjugates, antibody conjugates, and antibody fragment conjugates.

3. A method according to claim 1, wherein said protein conjugate is a radiolabeled conjugate.

4. A method according to claim 3, wherein the radiolabel in said radiolabeled conjugate is an imaging isotope.

5. A method according to claim 3, wherein the radiolabel in said radiolabeled conjugate is an therapeutic isotope.

6. A method according to claim 1, wherein said protein conjugate is selected from the group consisting of radiolabeled hapten conjugates and haptens conjugated to a cytotoxic agent.

7. A method according to claim 1, wherein said protein conjugate comprises a cytotoxic agent.

8. The method according to claim 1, wherein D-lysine is administered to said patient.

9. The method according to claim 1, wherein poly-D-lysine is administered to said patient.

11. The method according to claim 1, wherein a mixture of at least two of said compounds is administered to said patient.

12. The method according to claim 1, wherein said poly-lysine has a molecular weight of 15-30 kD.

13. The method according to claim 1, wherein said compound is parenterally administered to said patient in a physiologically acceptable aqueous solution.

14. The method according to claim 13, wherein said physiologically acceptable aqueous solution is administered to said patient by continuous infusion.

15. The method according to claim 13, wherein said physiologically acceptable aqueous solution is administered to said patient by means of at least one injection of a bolus of said solution.

16. The method according to claim 15, wherein said physiologically acceptable aqueous solution is administered to said patient by means of at least one injection of a bolus of said solution followed by oral administration in a physiologically acceptable carrier.

17. The method according to claim 1, wherein said compound is orally administered to said patient in a physiologically acceptable carrier.

18. A method of reducing kidney retention of a protein conjugate in a patient undergoing treatment with a targeting protein conjugate comprising administering to said patient, one or more compounds selected from the group consisting of D-lysine, poly-lysine having a molecular weight in the range 1-60 kD, pharmaceutically acceptable salts thereof and carboxyl derivatives thereof, wherein said protein conjugate has a molecular weight that is not greater than about 60 kD,

wherein the pharmaceutically acceptable salt and carboxyl derivative of poly-lysine has a molecular weight in the range 1-60 kD,

whereby said compound or compounds reduce kidney retention of said conjugates.

19. A method according to claim 18, wherein said protein conjugate is selected from the group consisting of peptide conjugates, polypeptide conjugates, glycoprotein conjugates, lipoprotein conjugates, antibody conjugates, and antibody fragment conjugates.

20. A method according to claim 18, wherein said targeting protein conjugate comprises a ribonucleic acid binding protein.

21. A method according to claim 20, wherein said ribonucleic acid binding protein is a ribonuclease.

23. A method according to claim 18, wherein said protein conjugate is a radiolabeled conjugate.

24. A method according to claim 23, wherein the radiolabel in said radiolabeled conjugates is an imaging isotope.

25. A method according to claim 23, wherein the radiolabel in said radiolabeled conjugates is a therapeutic isotope.

26. A method according to claim 18, wherein said protein conjugate is selected from the group consisting of radiolabeled hapten conjugates and haptens conjugated to a cytotoxic agent.

27. A method according to claim 18, wherein said protein conjugate comprises a cytotoxic agent.

28. The method according to claim 18, wherein D-lysine is administered to said patient.

29. The method according to claim 18, wherein poly-D-lysine is administered to said patient.

31. The method according to claim 18, wherein a mixture of at least two of said compounds is administered to said patient.

32. The method according to claim 18, wherein said poly-lysine has a molecular weight of 15-30 kD.

33. The method according to claim 18, wherein said compound is parenterally administered to said patient in a physiologically acceptable aqueous solution.

34. The method according to claim 33, wherein said physiologically acceptable aqueous solution is administered to said patient by continuous infusion.

35. The method according to claim 34, wherein said physiologically acceptable aqueous solution is administered to said patient by means of at least one injection of a bolus of said solution.

36. The method according to claim 35, wherein said physiologically acceptable aqueous solution is administered to said patient by means of at least one injection of a

bolus of said solution followed by oral administration in a physiologically acceptable carrier.

37. The method according to claim 18, wherein said compound is orally administered to said patient in a physiologically acceptable carrier.

38. In a cancer therapeutic or diagnostic method comprising administering to a patient in need thereof a protein conjugate comprising a cytotoxic agent or an imaging isotope, wherein said protein conjugate has a molecular weight that is not greater than about 60 kD, the improvement comprising additionally administering to said patient one or more compounds selected from the group consisting of D-lysine, poly-lysine having a molecular weight in the range 1-60 kD, pharmaceutically acceptable salts thereof and carboxyl derivatives thereof, to reduce kidney retention of said cytotoxic agent or imaging isotope.

39. The method according to claim 1, wherein poly-L-lysine is administered to said patient.

40. A method according to claim 21, wherein said ribonuclease is an ONCONASE®.

41. The method according to claim 18, wherein poly-L-lysine is administered to said patient.

IX. EVIDENCE APPENDIX

The following is a list of references entered by the Examiner and/or relied upon by Appellant in this appeal, along with a statement setting forth where in the record that evidence was entered by the examiner and/or the appellant. Copies of each piece of evidence are provided herewith.

Reference	Location in the Record
1. Behr *et al.*., *Cancer Research* 55, 3825-2834, September 1, 1995.	Non-Final Office Action of 10/21/2004 (pages 8-9); Final Office Action of 06/07/2005 (page 5).
2. Gray *et al.* (U.S. Patent No. 5,380,513).	Non-Final Office Action of 10/21/2004 (pages 9-11); Final Office Action of 06/07/2005 (page 5).
3. Raines *et al.* (U.S. Patent No. 5,840,296).	Non-Final Office Action of 10/21/2004 (pages 9-11); Final Office Action of 06/07/2005 (page 5).
4. Pandrol USA, LP *et al.*, v. Airboss Railway Products, Inc. *et al.*, (Slip. Op. 04-1069) (September 19, 2005).	Appeal Brief
5. Behr *et al.* (U.S. Patent No. 5,843,894).	Non-Final Office Action of 06/10/1997 (page 3); Final Office Action of 06/07/2005 (pages 3-5).
6. Non-Final Office Action of 10/21/2004	

X. RELATED PROCEEDINGS APPENDIX

There are no related proceedings.

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Reduction of the Renal Uptake of Radiolabeled Monoclonal Antibody Fragments by Cationic Amino Acids and Their Derivatives[1]

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ABSTRACT

The renal uptake of radiolabeled antibody fragments and peptides is a problem in radioimmunodetection and radioimmunotherapy, especially with intracellularly retained radiometals. The aim of this study was to develop suitable methods to reduce this kidney uptake. BALB/c mice or nude mice bearing the human GW-39 colon carcinoma xenograft were given i.p. injections of basic amino acids or a range of different basic amino acid derivatives, amino sugars, as well as cationic peptides. The effect of these agents on the biodistribution of Fab' and F(ab')$_2$ fragments of different mAbs radiolabeled with ^{99m}Tc, ^{188}Re, ^{111}In, ^{88}Y, or ^{125}I was studied. Tumor and organ uptake was determined and compared to untreated mice. The kidney uptake of Fab' fragments was reduced 5–6-fold in a dose-dependent manner as compared to untreated controls. The uptake in all other organs, as well as the tumor, was unaffected. A similar reduction in renal retention was seen for all other intracellularly retained isotopes, as well as for F(ab')$_2$ fragments. D- and L-isomers of lysine were equally effective whether given i.p. or p.o. D-glucosamine was effective, but its *N*-acetyl derivative was not. Basic polypeptides (*e.g.*, poly-L-lysine) were also effective; their potency increased with increasing molecular weight. HPLC of the urine taken from treated animals showed the excretion of intact Fab', in contrast to mostly low-molecular-weight metabolites in the control group. These studies indicate that a variety of basic compounds is capable of inhibiting the tubular reabsorption of peptides and proteins, thus lowering the kidney uptake of antibody fragments significantly. On a molecular basis, the effect seems to essentially rely on the presence of a positively charged amino group. By reducing renal retention of antibody fragments, their role as imaging and therapeutic agents may be expanded.

INTRODUCTION

The renal uptake of mAb fragments and peptides is a problem in RAID[4] or receptor scintigraphy of cancer or other diseases (1–3). It potentially compromises reliable diagnostic accuracy, especially in the retroperitoneal, periaortic, and epigastric regions. This effect is most apparent with intracellularly retained isotopes (^{111}In, ^{99m}Tc; Refs. 1,3). For ^{99m}Tc-labeled Fab' fragments, up to 25% of the injected dose is observed in the kidneys within 24 h (4). RAIT with antibody fragments conjugated to intracellularly retained radiometals (*e.g.*, ^{90}Y, $^{186/188}$Re, ^{67}Cu, ^{177}Lu) may be limited by elevated renal retention (5). Therapeutic trials with radiometal-labeled Fab' fragments or receptor-binding peptides have not been reported until now, presumably because of a lower absolute tumor uptake (4) but also because of their anticipated kidney radiotoxicity (5).

Renal accretion of peptides and small proteins is likely a consequence of glomerular filtration of molecules smaller than M_r 60,000, followed by their tubular reabsorption and lysosomal degradation (4, 6–8). Whereas iodine is released quickly from cells, radiometals would be retained, bound to ubiquitous intracellular metal-binding proteins (9). The kidney is well known as major site in the catabolism of low-molecular-weight proteins (4, 6–8). Basic amino acids, such as L-lysine and L-arginine, when administered in high doses, are able to induce functional proteinuria (Ref. 6; for review, see Ref. 7). Two previous studies suggested that L-lysine is effective in decreasing the renal uptake of radiolabeled peptides. Hammond *et al.* (11) studied the effect of an amino acid solution on the renal uptake of the ^{111}In-labeled somatostatin analogue, octreotide, in patients. Although the effect was clearly demonstrated, their analysis was only semiquantitative. Pimm *et al.* (12) reported on the effect of repeated i.p. injections of high doses of L-lysine on the renal uptake of ^{111}In-labeled Fab' fragments in BALB/c mice. They showed a highly significant reduction in renal uptake. However, none of these studies evaluated the effectiveness on radiolabels other than ^{111}In, the molecular characteristics of the substance enabling it to reduce the renal uptake, its (patho-) physiological mechanisms, or the effectiveness on different types of immunoglobulin subclasses, as well as on the uptake of larger protein molecules (*e.g.*, F(ab')$_2$); the latter is especially of therapeutic interest. Because its molecular weight is above the renally filterable size (8), the mechanism of renal uptake of F(ab')$_2$ is still poorly understood (10). Furthermore, no data on the effect of such a procedure on tumor uptake have been reported.

In this study, we present our results on reducing the renal uptake of a variety of radiometals, as well as iodine, bound to Fab' or F(ab')$_2$ fragments. We analyze the possible physiological mechanism of this uptake reduction, discuss the common molecular characteristics of the effective agents and their route of administration, and report dosimetric values that support possible therapeutic applications. These findings have appeared in abstract form (13–15).

MATERIALS AND METHODS

Antibodies. Several murine mAbs were used in these studies, including the anti-CEA mAbs NP-4 and MN-14 (16,17), the anti-colon-specific antigen p mAb Mu-9 (Ref. 18; all three of IgG1 subtype), and the anti-B-cell lymphoma (anti-CD22) antibody LL2 (Ref. 19; IgG$_{2a}$ subtype). All antibodies were purified from mouse ascites by protein A and ion exchange chromatography on S- and Q-Sepharose (Pharmacia, Piscataway, NJ). Purity was checked by immunoelectrophoresis, SDS-PAGE, and isoelectric focusing. The isoelectric points of the antibodies used for this study were 6.6–6.9 for MN-14 (IgG and fragments); 5.7–6.1 for NP-4 IgG, 5.3–5.8 for its fragments; 7.1–7.9 for LL2 IgG, 7.8–8.7 for its fragments; and 5.3–5.8 for Mu-9 and its fragments.

Fragmentation and Conjugation of Antibodies. F(ab')$_2$ fragments of the NP-4, Mu-9, and LL2 mAbs were prepared by pepsin digestion, whereas F(ab)$_2$ fragments of MN-14 were prepared by papain digestion. Each was then purified by protein A chromatography and exhaustive ultrafiltration. The bivalent fragments were reduced to their corresponding monovalent fragments by reduction with cysteine, as described earlier (20). Fab' fragments of NP-4 and LL2 were prepared for ^{99m}Tc labeling by following the "instant kit"

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[4] The abbreviations used are: RAID, radioimmunodetection; RAIT, radioimmunotherapy; CEA, carcinoembryonic antigen; SCN-Bz-DTPA, isothiocyanate benzyl-diethylene-triamine-pentaacetate; MTD, maximum tolerated dose; % ID/g, percent of injected dose per gram; p.i., postinjection.

formulation of Hansen *et al.* (21). For ^{188}Re labeling, SH derivatives of Fab' fragments of Mu-9 and LL2 were prepared by reduction with 2–20 μM 2-mercaptoethanol for 10 min at 4°C, as described previously (22). For ^{111}In and ^{88}Y labeling, SCN-Bz-DTPA conjugates of MN-14 $F(ab)_2$ and iodoacetamide-blocked Fab, as well as LL2 $F(ab')_2$, were prepared by adding SCN-Bz-DTPA to the antibody (5.0 mg/ml), previously dialyzed against 100 mM HEPES buffer (pH 8.6), containing 150 mM NaCl, at a 8:1 molar excess of DTPA to mAb. After overnight incubation at room temperature, the antibody conjugates were purified from unreacted SCN-Bz-DTPA by gel filtration chromatography on a 1- x 50-cm column of Sephadex G-50 (Pharmacia, Piscataway, NJ).

Radiolabeling. Technetium-99m was obtained from a ^{99}Mo/^{99m}Tc generator system (Syncor, Fairfield, NJ) as a solution of sodium pertechnetate in 0.9% sodium chloride. Rhenium-188 was obtained from an in-house tungsten-188 (^{188}W/^{188}Re) generator system (Oak Ridge National Laboratory, Oak Ridge, TN) as a solution in 0.9% sodium chloride (22). Indium-111 was purchased as $^{111}InCl_3$ in 0.05 M HCl from DuPont New England Nuclear (Billerica, MA), as well as iodine-125 as sodium iodine in 10^{-5} M NaOH. Yttrium-88 was obtained as $^{88}YCl_3$ in 6 M HCl from the Los Alamos National Laboratory (Los Alamos, NM).

Radioiodination was performed with ^{125}INa using the iodogen method, as described previously (23). ^{99m}Tc and ^{188}Re labeling were performed by reconstituting the antibody kits with sodium pertechnetate or perrhenate in 0.9% NaCl (21,22). Labeling with ^{111}In and ^{88}Y followed procedures described earlier (24).

All labeled antibodies were administered within 3 h of their preparation. The quality of each preparation was tested by instant TLC and HPLC (Bio-Sil SEC-250 column, 300 x 7.8 mm; Bio-Rad Laboratories, Richmond, CA), as well as by measuring its immunoreactivity. No aggregates were detectable, the amount of unbound isotope was less than 5%, and 75–80% of the radiolabeled antibodies bound to the respective immunoadsorbent column in each preparation. In ^{99m}Tc-labeled Fab' fragment preparations, the amount of residual $F(ab')_2$ varied between 2 and 20% of the total activity in different experiments.

Animal Models and Biodistribution Studies. Female BALB/c mice, 19–22 g and 4–5 weeks of age (Harlan, Indianapolis, IN), were used as non-tumor-bearing animals. The human colon carcinoma transplant GW-39 (25) was grown s.c. in 5–6-week-old female athymic mice (Harlan, Indianapolis, IN). The animals given i.v. injections in the tail vein with approximately 5–10 μg of antibody fragment protein (*i.e.*, 25–40 μCi of ^{99m}Tc, ^{188}Re, or ^{111}In; 4 μCi of ^{88}Y; and 10 μCi of ^{125}I). In several studies, animals were co-injected with a mixture of ^{111}In- and ^{88}Y-labeled mAbs. In these instances, windows were set for each radionuclide, and the backscatter of ^{88}Y into the ^{111}In window was corrected. The mice were necropsied typically at 4 and 24 h for ^{99m}Tc, and at 4, 24, 72, 96, and 168 h for ^{125}I-, ^{111}In-, ^{88}Y-, and ^{188}Re-labeled antibodies. They were first anesthetized with sodium pentobarbital and bled by cardiac puncture. After cervical dislocation, the animals were dissected. The amount of activity in the tumors and tissues (liver, spleen, kidney, lung, intestine, blood, and bone) was determined by γ scintillation counting using an injection standard to account for physical decay. The number of animals used for each study is indicated in the results (typically five animals/group at each time point). The radiation dose to the tissues was calculated from the biodistribution data, as published previously (26).

Kidney Uptake Reduction. All the chemical compounds were obtained from Sigma Chemical Co. (St. Louis, MO). L-lysine, D-lysine, and L-arginine (all as monohydrochloride salts) were dissolved in PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride; pH 7.4) at 160 mg/ml. L-lysine ethyl ester dihydrochloride was dissolved in PBS to yield a concentration of 80 mg/ml. Poly-L-lysine, in a molecular weight range between M_r 1000 and 4000, as well as M_r 15,000–30,000, was used as the hydrochloride salt in solutions of 25 mg/ml and 10 mg/ml, respectively. L-tyrosine, L-phenylalanine, and L-cystine (free bases) were each used as saturated suspensions (160 mg/ml) in PBS. L-glutamate (monosodium salt) was dissolved in PBS at a concentration of 150 mg/ml. D-glucosamine hydrochloride and its *N*-acetylated derivative were in PBS at a concentration of 80 mg/ml.

The animals were injected with the amino acid, peptide, or amino sugar solutions i.p. or i.v. in amounts and frequency indicated in "Results." For example, 20-g animals that were given 4 × 2000 μg/g of L-lysine received 4 i.p. injections, each totaling 40 mg in 0.25 ml. Control animals were given injections of the same volume of PBS or 0.9% saline. For oral administration, D- and L-lysine monohydrochloride salts were dissolved in PBS at a concentration of 200 mg/ml. Five hundred μl of these solutions were administered p.o. to BALB/c mice through an oral intubation catheter 30 min before the antibody injection. Statistical analysis was performed with the Student's *t* test.

Study Design. In a pilot experiment, the normal kidney and organ uptake kinetics of ^{99m}Tc-labeled Fab' fragments were studied in BALB/c mice. In a second experiment, the effect of L-lysine on the biodistribution was examined in BALB/c mice, given in various doses and administration schedules (i.p., i.v., and p.o., see "Results"). To exclude effects on the tumor uptake, similar studies were carried out with GW-39 tumor-bearing nude mice. To address the question whether the effects of lysine are a general principle for all sorts of radiolabels, its influence on the biodistribution of ^{188}Re, ^{111}In, ^{88}Y, and ^{125}I-labeled Fab' fragments was studied in tumor-bearing nude mice. The requirements of a compound, enabling it to reduce the renal uptake, were assessed on a molecular level by examination of a range of different amino acids, their derivatives, as well as amino sugars and polypeptides. To elucidate their mechanism of action, the urine and sera of treated animals and controls were analyzed by size exclusion HPLC. Finally, the applicability of this methodology to ^{125}I-, ^{111}In-, and ^{88}Y-labeled $F(ab')_2$ fragments was studied.

RESULTS

Renal Uptake Kinetics of Radiolabeled Fab'. Fig. 1 shows the typical kinetics of ^{99m}Tc-labeled NP-4 Fab' in BALB/c mice. ^{99m}Tc-labeled Fab' has a rapid renal uptake that reaches its maximum 2 h after the i.v. antibody injection, with a plateau until 4 h p.i., after which the excretion of the retained activity predominates. A considerable interexperimental variability was observed in the percent uptake in the kidneys that was not apparent in the other organs and tumor. In 10 separate experiments conducted with ^{99m}Tc-NP-4 Fab', the peak kidney uptake ranged from an average of 65.0 ± 10.9% ID/g to as high as 122.6 ± 20.8% ID/g (mean, 88.1% ID/g), accounting for an absolute uptake in both kidneys of 18–35% of the injected activity per organ. No correlation was found between the variability in this renal uptake and the content of residual $F(ab')_2$ or unbound ^{99m}Tc in the initial labeled product before injection. Thus, to correct for the variability in renal uptake, every study was controlled internally by including an untreated group of animals for each experiment. All values for the reduction in renal retention given below refer, therefore, to this internal control of the individual experiment. However, the intraexperiment variability was considerably lower, and even the use

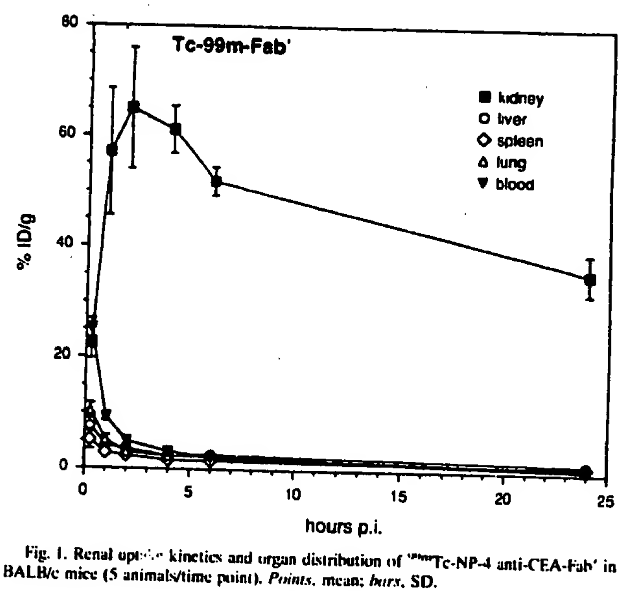

Fig. 1. Renal uptake kinetics and organ distribution of ^{99m}Tc-NP-4 anti-CEA-Fab' in BALB/c mice (5 animals/time point). *Points*, mean; *bars*, SD.

of pooled control data would not affect the qualitative results of the study.

Effect of L-lysine on the Renal Retention of Fab′ Fragments. Fig. 2 shows the dose-effect relationship between L-lysine administered i.p. and the kidney uptake at 4 and 24 h after the administration of ^{99m}Tc-NP-4 Fab′ in BALB/c mice. L-lysine was injected i.p. 4 times, starting from 30 min before the labeled antibody was given, and then at approximately hourly intervals for 3 h (*i.e.*, −30 min and 1, 2, and 3 h p.i.). At a total dose below 4 × 100 μg/g body weight, no significant effect on renal uptake was observed. Above this threshold, a strong dose-effect relationship was found. At 4 × 2000 μg/g, the kidney uptake was reduced by more than 5-fold, to only 19.0 ± 5.7% ID/g from 115.1 ± 6.4% ID/g in the control animals ($P < 0.001$). No statistically significant effect was observed on the uptake in any other organ. However, in some experiments, a tendency toward a slightly enhanced blood clearance was noticed with lysine challenge independent of the actual lysine dose. For example, at 4 h p.i., the % ID/g in the blood was 1.85 ± 1.15 with 4 × 1000 μg/g of L-lysine *versus* 3.72 ± 0.35 in the control group ($P < 0.01$). The MTD of L-lysine was reached at 4 × 2500 μg/g, and beyond this dose level, no additional reduction was seen in renal retention. This dose level was tolerated without any obvious short- or long-term effects. Animals observed for over 3 months behaved normally, and at necropsy there were no obvious changes in any organs, especially the renal histology. However, no formal toxicology studies were performed. At doses exceeding 4 × 2500 μg, the mice became lethargic and developed fluid in the body cavities (*e.g.*, pleural effusions). The fluid build-up in the body cavities may have been due to the need to challenge with more than 1 ml of a highly hypertonic solution per animal, thus causing a fluid overload. These volumes were required because higher concentrated lysine solutions could not be generated due to its limited solubility.

By 24 h after injecting ^{99m}Tc-Fab′, renal uptake in the untreated animals had decreased to approximately one-fourth of the original uptake. Animals treated initially with lysine continued to show a persistent reduction in renal uptake (Fig. 2, *bottom*). For example, animals initially treated with 4 × 2000 μg of L-lysine had only 7.5 ± 3.5% ID/g in the kidneys at 24 h, compared to 34.0 ± 4.4% ID/g in the controls ($P < 0.001$). Almost identical results as for ^{99m}Tc-labeled Fab′ NP-4 (derived from an IgG1) were achieved for ^{99m}Tc-Fab′ LL2 (IgG2a subtype) under lysine treatment (data not shown).

Oral administration of L-lysine was equally effective in reducing renal uptake. The kidney uptake of ^{99m}Tc-labeled NP-4 Fab′ fragments was reduced by 80.5% using a single p.o. dose of 5 mg L-lysine/g of body weight given 30 min before the antibody injection. This compared favorably to the 72.4% seen in a parallel group of animals given four i.p. injections (*i.e.*, 4 × 2000 μg/g).

Similar studies were carried out in nude mice bearing human colonic tumor xenografts to determine whether tumor targeting was affected. Lysine treatment had no significant influence on tumor uptake (3.5 ± 0.6 *versus* 3.2 ± 0.5% ID/g at 4 h p.i., and 1.7 ± 0.2 *versus* 1.9 ± 0.5% ID/g at 24 h p.i. in controls *versus* treated mice, respectively; $P > 0.2$). Uptake in all organs other than the kidneys was also unaffected. By external scintigraphy, the reduced renal uptake improved visualization of the xenografted tumors, as well as normal organs near the kidneys (Fig. 3).

Encouraged by the highly effective single p.o. administration of L-lysine, less frequent i.p. injections were tested. If only a single injection of L-lysine at a dose of 2000 μg/g was given 30 min before the radioantibody injection, it was possible to reduce the renal uptake from 122.6 ± 20.8 to 69.1 ± 9.8% ID/g ($P < 0.001$). This is similar to the reduction level acheived by administering a total dose of 2000 μg/g in 4 fractions over 3 h (*i.e.*, 4 × 500 μg/g; see Fig. 2). Injection of 2000 μg/g twice (30 min before and 60 min after the radiolabeled antibody) reduced the uptake to 54.1 ± 6.0% ID/g, whereas the four injections reduced renal uptake to 19.0 ± 5.7% ID/g (*i.e.*, 15.5% of the controls). Injection of higher doses of the L-lysine solution at a single time point (*e.g.*, 8 mg/g once instead of 4 × 2 mg/g in hourly intervals) was not possible because of the above-mentioned toxic effects of higher single doses (causing lethargy and effusions in the body cavities).

L-Lysine had a similar effect on reducing renal retention for ^{188}Re-labeled Mu-9 Fab′ (Fig. 4). Lysine treatment (4 × 2000 μg/g) reduced kidney uptake to 29% of the untreated controls after 4 h p.i. It was interesting to note that in both groups, the controls and the lysine-treated animals, the release of the tubularly retained ^{188}Re was at a similar rate after 24 h (*i.e.*, parallel curves for % ID/g in the kidneys for controls and treated mice). Apparently, there is no significant effect on the liberation of isotope after it has been taken up. Indeed, prolongation of lysine administration over longer periods of time or administration starting at 4 h after the antibody administration did not decrease the kidney retention of already tubularly stored activity (^{99m}Tc- or ^{188}Re-Fab′) in comparison to the control group (data not shown). As observed with ^{99m}Tc-labeled fragments, no effect on the uptake in the tumor or other organs was seen with ^{188}Re-labeled fragments. The only exception was the blood clearance, which was minimally enhanced [*e.g.*, 1.99 ± 0.29 *versus* 1.50 ± 0.25% ID/g at

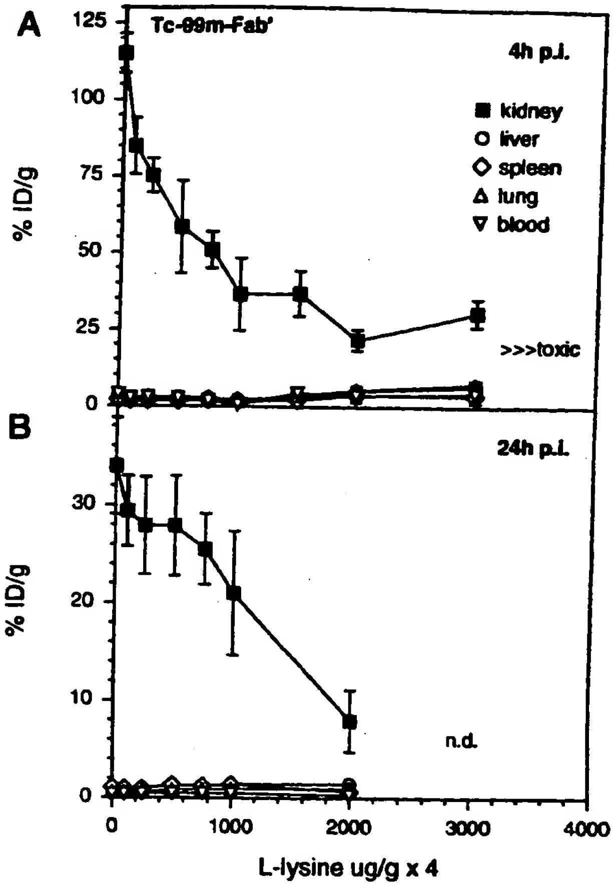

Fig. 2. Dose-effect relationship between L-lysine hydrochloride, administered i.p. at hourly intervals, on the renal uptake of ^{99m}Tc-NP-4 Fab′ fragments in BALB/c mice. *A*, 4 h after radioantibody injection; *B*, 24 h after antibody injection (5 animals each/time point). *Points*, mean; *bars*, SD.

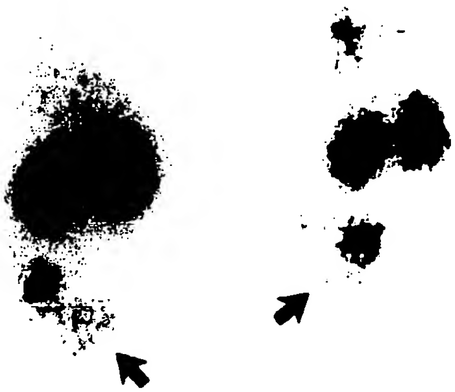

Fig. 3. External scintigraphy of human GW-39 colon carcinoma-bearing nude mice 4 h after injection of ^{99m}Tc-NP-4 Fab′ (Sopha DS-7 γ camera, equipped with a high-energy pinhole collimator; 100 kilocounts were acquired per frame). The animal on the *left* was untreated; the animal on the *right* received 4 × 2000 μg/g L-lysine i.p. Renal uptake under lysine treatment is significantly reduced, allowing better visualization of the organs near the kidneys, including the tumor (*arrows*).

4 h p.i. in controls and treated animals (n = 5 each; P < 0.05)], respectively.

Table 1 summarizes the dosimetry of ^{188}Re-Mu-9 Fab′ in control and lysine-treated animals. The kidney dose under lysine treatment is approximately 39% of that in the control group, whereas no marked effect on the tumor dose could be observed. The slightly decreased blood dose may reflect a minimally enhanced clearance, which was also observed with ^{99m}Tc-Fab′.

To test the effectiveness of L-lysine in reducing kidney uptake of indium- and yttrium-labeled Fab fragments, nude mice bearing GW-39 tumor xenografts were injected with a mixture of ^{111}In- and ^{88}Y-labeled anti-CEA MN-14 Fab. Treated animals received different amounts of L-lysine up to 4 × 2000 μg/g, whereas a parallel group of animals remained untreated. Fig. 5 and Table 2 summarize the results. Lysine treatment reduced the kidney uptake of ^{111}In-Fab to 24.1, 22.0, 22.8, and 20.5% of the controls after 4, 24, 72, and 168 h, respectively. A similar percent reduction was achieved for ^{88}Y-Fab (*e.g.*, 25.2, 24.8, 22.3, and 25.9% of the controls at 4, 24, 72, and 168 h, respectively). The initial amount of ^{88}Y-Fab in the kidneys was lower than the amount of the ^{111}In-Fab (48.7 ± 8.2 *versus* 72.1 ± 5.8% ID/g at 4 h p.i.; P < 0.001). The ^{88}Y uptake in the other organs was also approximately 30% lower than the respective ^{111}In retention values, except for the bone, where the retention of ^{88}Y was slightly higher than that of ^{111}In (data not shown).

Previous studies showed that iodinated Fab′ is also taken up in the kidneys but liberated more quickly than the other isotopes tested (5). Studies were undertaken to determine whether lysine treatment would be of any benefit to iodinated Fab′. ^{125}I-labeled anti-CEA NP-4 Fab′ was given to animals with and without lysine treatment. Although the kidney uptake of iodine-labeled Fab′ in untreated animals was much lower than that observed for all radiometals (*e.g.*, at 4 h p.i. 3.6 ± 0.2 *versus* 122.6 ± 20.8% ID/g for ^{125}I- and ^{99m}Tc-NP-4 Fab′, respectively; P < 0.001), lysine treatment still reduced the renal uptake to 38.7% of the untreated controls at 4 h p.i (1.4 ± 0.3% ID/g; P < 0.05). The absorbed dose to the kidney was decreased by 50% when calculated for a ^{131}I-labeled Fab′ (data not shown).

Mechanism of Action. BALB/c mice were given ^{99m}Tc-NP-4 Fab′ with or without lysine treatment. Serum and urine taken from the animals at 4 h p.i. were analyzed by size-exclusion HPLC (Fig. 6). No significant differences were found in the molecular composition of labeled components in the blood (data not shown). For example at 4 h p.i., 23% of the total serum activity was in the form of $F(ab')_2$, 74% in the form of Fab′ fragments, and less than 3% was in the form of low-molecular-weight compounds. The majority of the radioactivity in the urine of the control group was of low molecular weight, with only traces of activity (less than 5%) in the original M_r 50,000 range. However, 65% of the radioactivity in the urine of lysine-treated mice coeluted with native ^{99m}Tc-Fab′. Eighty-one % of the activity isolated from this peak also bound to a CEA immunoadsorbant, further suggesting that it was intact Fab′ fragments. These data suggest that lysine inhibits the tubular reabsorption of glomerularly filtered Fab′. As a consequence, nonmetabolized Fab′ is excreted, whereas in untreated animals, the filtered protein is reabsorbed and lysosomally degraded in the tubular cells.

Other Agents. The stereospecificity of this effect was assessed by examining other substances that are capable of blocking the tubular reabsorption of antibody fragments. D-Lysine was tested under the same conditions as its L-isomer, both as an i.p. injection (different amounts up to 4 × 2000 μg/g) and by p.o. adminstration (1 × 5 mg/g). No significant differences in the dose-effect relationship could be observed between both steric isomers when given i.p. (Fig. 7). It was surprising that p.o. administered D-lysine reduced renal uptake to a level 64.5% less than the control animals, compared to 72.5% for i.p.-injected D-lysine.

Another basic amino acid, L-arginine, was tested. Arginine hydro-

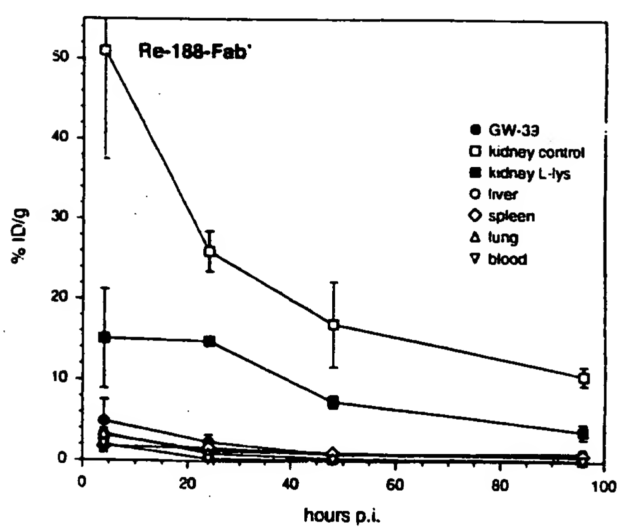

Fig. 4. Renal uptake and organ kinetics of ^{188}Re-labeled anti-colon-specific antigen p Mu-9 Fab′ in GW-39 tumor-bearing nude mice with and without L-lysine (4 × 2000 μg/g i.p.) treatment. Refer to Table 1 for tumor and organ dosimetry. *Points*, mean; *bars*, SD.

Table 1 *Dosimetry of ^{188}Re-Mu-9 Fab' in nude mice bearing GW-39 tumors with and without four i.p. injections of 2000 μg L-lysine/g body weight*

	Control		L-Lysine		Dose ratio (lysine:control)
	cGy/mCi	T:NT[a]	cGy/mCi	TN:T[a]	
GW-39	620.3		763.4		1.23
Liver	611.3	1.01	587.6	1.30	0.96
Spleen	306.0	2.03	289.0	2.64	0.94
Kidney	7818.8	0.08	3085.2	0.25	0.39
Lung	537.7	1.15	410.4	1.86	0.76
Blood	256.4	2.42	203.1	3.76	0.79
Bone	120.9	5.13	107.3	7.11	0.89

[a] Tumor:non-tumor (T:NT) ratios in this and the following tables express the tumor to normal organ radiation dose ratios for the organs given in the respective line.

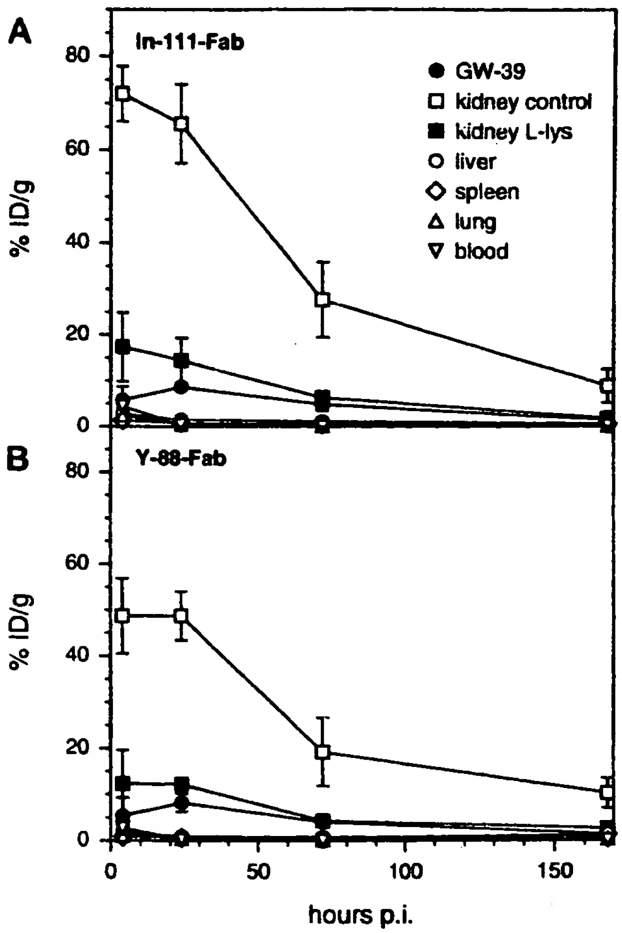

Fig. 5. Renal and organ uptake of ^{111}In (*A*) and ^{88}Y-Bz-DTPA MN-14 Fab (*B*) in GW-39 tumor-bearing nude mice with and without L-lysine (4 × 2000 μg/g i.p.) treatment. Refer to Table 2 for tumor and organ dosimetry. *Points*, mean; *bars*, SD.

chloride was also effective but, on a weight basis, to a lower extent than lysine (Fig. 7). Therefore, its effectiveness was not tested at higher doses. L-Lysine ethyl ester was more effective, on a weight-basis, than underivatized lysine, but injections were necessary for maximal effectiveness (presumably because of a rapid whole-body clearance of substances of such low molecular weight).

In contrast to the basic amino acids and their derivatives, glutamate (as an example of an anionic amino acid), phenylalanine, and tyrosine (as neutral amino acids), as well as administration of the same volumes of PBS or saline as used for lysine administration, were ineffective in reducing the kidney uptake of radiolabeled antibody fragments (data not shown).

To assess further the requirements of a substance capable of blocking tubular protein reabsorption on a molecular level, D-glucosamine was examined. As shown in Fig. 7, this amino sugar was almost as effective in reducing the kidney uptake as was the amino acid lysine. In contrast, its uncharged *N*-acetyl derivative was completely inactive.

Polymeric substances of higher molecular weight were also investigated to determine whether these substances would either improve the effect or reduce the number of injections necessary to reach maximal effectiveness (Fig. 8). Poly-L-lysine, with a molecular weight range between M_r 1000 and 4000, was able to reduce the kidney uptake with a single i.p. injection in significantly lower doses than the monomer. Fifty % reduction was observed at doses of approximately 1 × 200 μg/g. To obtain a similar reduction with L-Lysine, 4 × 750 μg/g was required. However, the MTD for poly-L-lysine (M_r 1000–4000) was reached at 300 μg/g, so that the maximum uptake reduction achieveable with poly-L-lysine will be less than with the monomer. The potency of poly-L-lysine increased with increasing molecular weight. The polymer in a size range between M_r 15,000 and 30,000 showed a 50% uptake reduction at 1 × 20 μg/g. This dose was also shown to be its MTD. Despite the fact that higher doses were not possible, there also seemed to be a saturation at approximately a 40–50% uptake reduction (Fig. 8). When given twice at 30 min before and 1 h after antibody administration, kidney uptake was the same as when a single injection was given. Thus, there was no apparent advantage for using these polymeric substances other than achieving a similar reduction with a single injection. Similar effectiveness and

Table 2 *Effect of L-lysine treatment (4 × 2000 μg/g i.p.) on the ^{90}Y dosimetry of anti-CEA MN-14 Fab (based on the ^{88}Y biodistribution) in GW-39 tumor-bearing nude mice*

	Control		L-Lysine		Dose ratio (lysine:control)
	cGy/mCi	T:NT[a]	cGy/mCi	T:NT	
GW-39	4953.8		5751.1		1.16
Liver	1339.9	3.70	1651.9	3.48	1.23
Spleen	626.9	7.90	499.2	11.52	0.80
Kidney	33057.2	0.15	6231.0	0.93	0.18
Lung	385.6	12.85	431.1	13.30	1.12
Blood	593.6	8.35	664.1	8.66	0.95
Intestine	519.9	9.53	560.2	10.26	1.08
Bone	493.2	10.04	588.4	9.77	1.19
Washed bone	396.7	12.49	486.8	11.81	1.23

[a] Tumor:nontumor ratio.

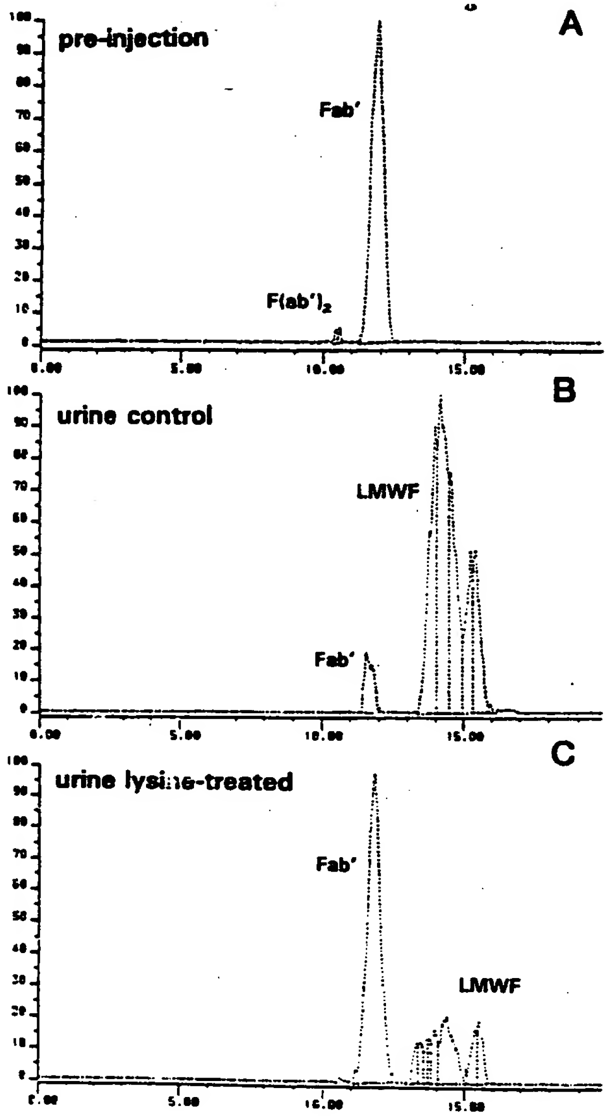

Fig. 6. Size-exclusion HPLC profiles of the urine of BALB/c mice treated with L-lysine (4×2000 μg/g i.p.) in comparison to controls. *A*, HPLC profile of the preinjection solution (^{99m}Tc-Fab' NP-4) containing 98% of the total activity bound to Fab' and 2% bound to residual $F(ab')_2$. *B*, urine of the controls; over 95% of excreted ^{99m}Tc is bound to low-molecular-weight metabolites (*LMWF*). *C*, urine of the lysine-treated mice; 65% of the activity is bound to intact Fab' (which retained an immunoreactivity of 81%).

toxicity was seen for protamine (Fig. 8) as an example of a naturally occurring basic polypeptide.

Combinations of lysine and polylysine were also tested. The optimal effect was found with two injections of a mixture of 2 mg/g lysine and 20 μg/g polylysine (M_r 15,000–30,000) at 30 min before and 1 h after antibody administration. With this administration regimen, the kidney uptake of ^{99m}Tc-labeled NP-4 Fab' was reduced to 32.5% of controls (31.7 ± 2.4% ID/g *versus* 97.7 ± 5.1% ID/g; $P < 0.001$). This is almost as effective as four i.p. injections of 2 mg/g of the monomer alone. Despite the fact that both compounds were given close to their individual MTDs, no acute or chronic toxicity was noticed over a 3-month period.

Reduction of Renal Uptake with $F(ab)_2$ Fragments. In untreated animals, kidney uptake of ^{111}In-labeled MN-14 $F(ab)_2$ was continuous over a period of 24 h (Fig. 9, *top*), with a significantly lower maximum uptake than that of ^{111}In-Fab fragments (49.7 ± 5.2 *versus* 72.1 ± 5.8; $P < 0.01$). After 24 h p.i., clearance from the kidneys prepondered. An almost identical biodistribution pattern was observed for ^{111}In-labeled $F(ab')_2$ fragments of the clone LL2.

The scheme of i.p. injections of L-lysine used for Fab' fragments (*i.e.*, −30 min and 1, 2, and 3 h) reduced the kidney uptake of $F(ab)_2$ fragments by about 50% of that seen in control animals (data not shown). Because the kinetics of $F(ab)_2$ accretion in the kidneys was slower than that of the Fab' (compare Figs. 1 and 9), the timing of the lysine treatments was extended to once every 2 h over a period of 8 h. This modification reduced the kidney uptake of $F(ab)_2$ fragments to the extent found with Fab' fragments. Similar to the experience with radioiodinated Fab', the uptake of ^{125}I-$F(ab)_2$ in control animals was lower than that seen for radiometal-labeled $F(ab)_2$. It was 3.2 ± 0.6% ID/g for ^{125}I-$F(ab)_2$ compared to ($P < 0.001$) 54.7 ± 11.2 and 43.9 ± 10.1% ID/g at 24 h p.i. for an ^{111}In- and ^{88}Y-$F(ab)_2$, respectively. Nevertheless, renal uptake was reduced for the ^{125}I-$F(ab)_2$ to approximately 60% of the control animals by the lysine treatment (data not shown). L-Lysine treatment reduced renal uptake (Fig. 9) by 70–80% to 16.5 ± 10.1% ID/g and 9.5 ± 5.9% ID/g at 24 h p.i. for the ^{111}In- and ^{88}Y-$F(ab)_2$, respectively ($P < 0.001$). The reduction in renal uptake lowered the absorbed dose to the kidneys for ^{90}Y-$F(ab)_2$ 4-fold (Table 3). Similar to the observations with ^{111}In- and ^{88}Y-labeled Fab fragments, there was a higher retention of ^{111}In-$F(ab)_2$,

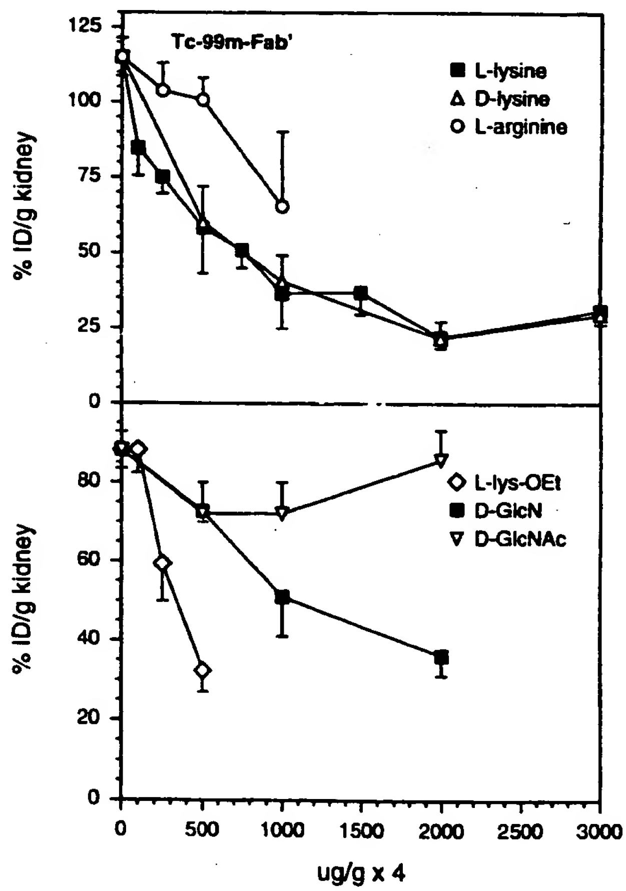

Fig. 7. Dose-effect relationship of L- and D-lysine, L-lysine ethyl ester, L-arginine, and D-glucosamine and its *N*-acetylated derivative, administered i.p. in hourly intervals, on the renal uptake of ^{99m}Tc NP-4 Fab'. *Points*, mean; *bars*, SD.

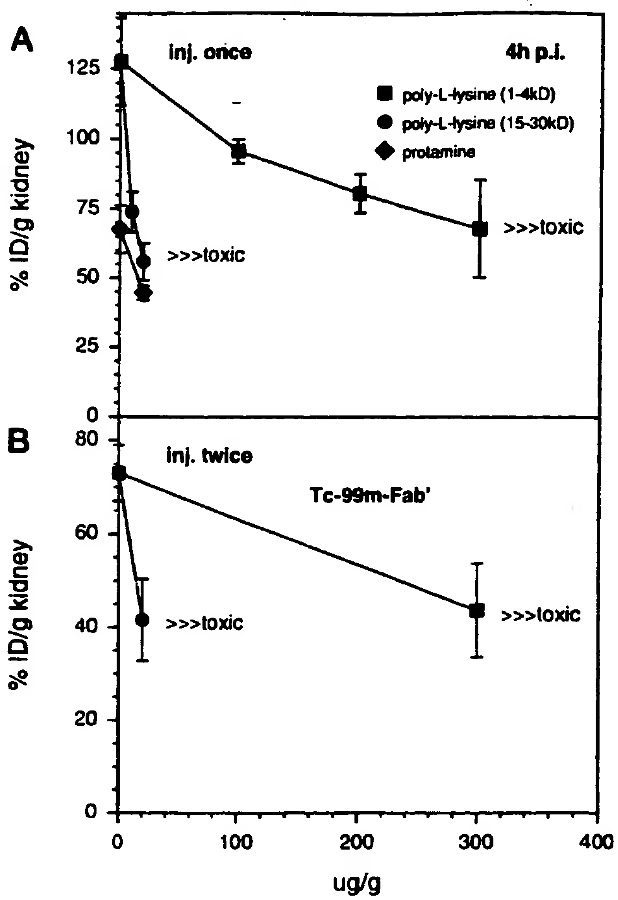

Fig. 8. Effect of basic peptides and proteins injected 30 min before (*A*) or 30 min before and 1 h after the radiolabeled antibody i.p. (*B*) on the renal uptake of ^{99m}Tc-labeled Fab' fragments of the anti-CEA antibody NP-4. *kD*, molecular weight in thousands. *Points*, mean; *bars*, SD.

when compared to the ^{88}Y-labeled fragment, leading to an overestimation of the actual ^{90}Y dose by indium (see above). As was observed with Fab' fragments, uptake reduction persisted for several days, and excretion of the retained radiometals paralleled the control group (Fig. 9). Similar results as for $F(ab)_2$ fragments of MN-14 (IgG1 subclass) were obtained for $F(ab')_2$ fragments of the anti-B-cell (CD22) clone LL2 (IgG2a isotype; data not shown).

HPLC analysis of the urine in the lysine-treated and control groups showed no intact $F(ab)_2$ or Fab fragments but only substances of low molecular weight (data not shown), indicating that the physiological mechanism of the $F(ab)_2$ catabolism is different from that of Fab'. However, lysine also can effectively block this tubular reabsorption.

DISCUSSION

Kidney uptake of proteins and peptides below the renally filterable size of approximately M_r 60,000 (28) can be a problem for RAID, sometimes obscuring abdominal lesions despite sufficient antibody uptake (1, 2, 29, 30). When used therapeutically, radiometal conjugates of antibody fragments or peptides will produce protracted renal retention that could make the kidneys the next dose-limiting organ after the hematopoietic system, if not the first dose-limiting organ itself. According to external beam irradiation data, the MTD for the kidneys is estimated as 2000 cGy (31). Above this level, the risk of radiation nephritis with subsequent scarring of glomeruli, nephrotic syndrome, and renal insufficiency rises considerably (31). Although no exact values are known for the tolerance of renal irradiation at lower dose rates as occur in RAIT, careful monitoring of renal function would be advised for radiometal-conjugated antibody fragments. Even for diagnostic applications with pure γ-emitters, such as ^{99m}Tc-Fab', the dose to the kidney can reach 0.42 ± 0.14 cGy/mCi (30–33). Thus, there are ample reasons for investigating methods that would reduce renal doses from radiolabeled anitbody fragments or peptides.

Others have attempted to reduce the kidney uptake of Fab' fragments by modification of the antibody itself, *e.g.*, by shielding their positive charges through *N*-acetylation of the antibody fragment (34). This approach was modeled after the findings that positive charges favor renal filtration and tubular accretion of polypeptides and proteins (8, 35). The success of this method, however, was limited and the immunoreactivity of the mAb fragment was compromised (34). Preliminary animal experimental results by Pimm *et al.* (12) and semiquantitative human data from Hammond *et al.* (11) suggested that L-lysine and some other basic amino acids are capable of reducing the kidney uptake of ^{111}In-labeled Fab' fragments and ^{111}In-labeled somatostatin analogues. Our study shows that the effect of lysine is not restricted to indium-labeled compounds but extends to all isotopes and antibodies tested. Thus, the effect is widely independ-

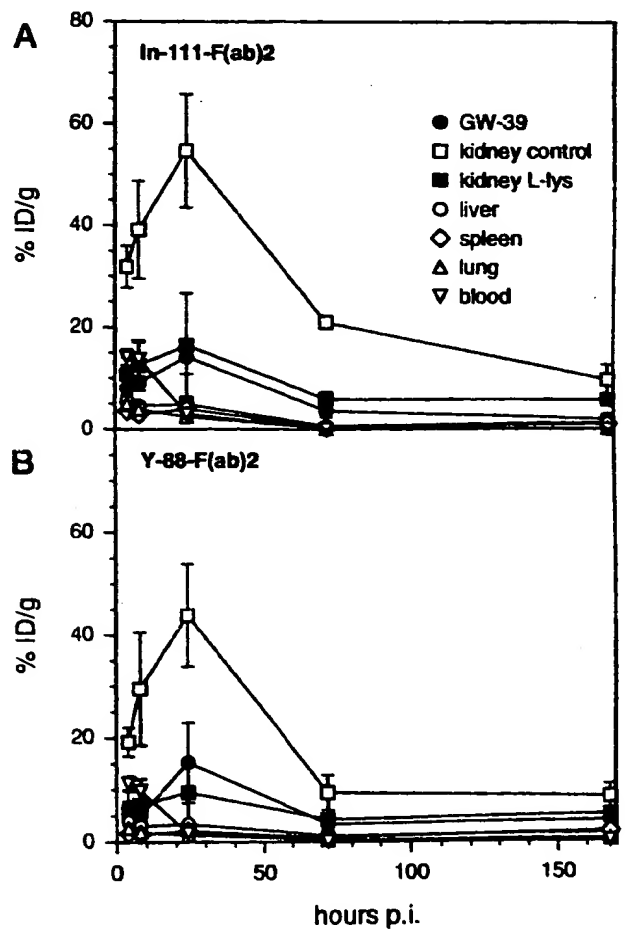

Fig. 9. Renal uptake reduction of ^{111}In- (*A*) and ^{88}Y-labeled $F(ab)_2$ (*B*) fragments of the anti-CEA antibody MN-14 in the time course with prolonged lysine administration (4 × 2000 μg/g every second hour i.p.). Refer to Table 3 for tumor and organ dosimetry. *Points*, mean; *bars*, SD.

Table 3 *Effect of treatment with L-lysine (4 × 2000 μg/g i.p. every second hour) on the ^{90}Y dosimetry based on the ^{88}Y biodistribution of anti-CEA MN-14 $F(ab)_2$ in GW-39 tumor-bearing nude mice*

	Control		L-Lysine		Dose ratio (lysine:control)
	cGy/mCi	T:NT[a]	cGy/mCi	T:NT	
GW-39	5875.9		6500.1		1.11
Liver	2333.7	2.52	3974.3	1.63	1.70
Spleen	737.7	7.96	848.6	7.66	1.15
Kidney	14892.9	0.39	3974.3	1.65	0.27
Lung	978.1	6.01	992.3	6.55	1.01
Blood	2795.4	2.10	2317.4	2.80	0.83
Intestine	695.9	8.44	757.5	8.58	1.09
Bone	569.9	10.31	786.9	8.26	1.38
Washed bone	656.6	8.95	476.8	13.63	0.73

[a] Tumor:nontumor ratio.

ent of the immunoglobuline subclass or other protein characteristics (*e.g.*, the pK_a value). As expected, the effect is more pronounced with intracellularly retained isotopes (radiometals) than with liberated ones (*e.g.*, iodine).

The molecular characteristics that enable a compound to be capable of inhibiting protein uptake seem to be very variable, provided that the substance carries a positive charge through an amino group. Basic amino acids (administered i.v., i.p., or p.o.), amino sugars, as well as basic peptides, are effective. The potency of the substances seems to increase with the amount of positive charges per molecule. For example, lysine ethyl ester, with its shielded negative carboxyl charge, is more potent than lysine itself, and the potency of polypeptides with lysyl residues rises with the molecular weight. The equal effectiveness of glucosamine, when compared to lysine, supports the concept that the effectivity of a compound essentially relies on the presence of a positively charged amino group. Accordingly, its *N*-acetylated derivative, lacking the positive charge, was not able to reduce renal accretion of Fab′ fragments.

It is known that high doses of basic amino acids can induce proteinuria (6, 7), presumably by blocking of tubular reabsorption of glomerularly filtered peptides (7), although several contradictory theories concerning the mechanism of action have been published (6, 7, 11, 12, 36). The excretion of essentially unchanged Fab′ in lysine-treated mice, in contrast to low-molecular-weight products in the controls, supports the theory of Morgenson and Sølling (6) that the major principle is inhibition of tubular reabsorption of primarily glomerularly filtered peptides. Although in this study, no attempts were made to quantitatively collect urine and feces of the animals (which is methodologically not trivial), the facts that no alteration in the uptake of any other organ was observed, unchanged Fab′ fragments were found qualitatively in the urine, and external whole-body scintigraphy showed no changes in biodistribution in any part of the body other than the kidneys, clearly indicate that the mechanism relies on simple urinary excretion of the protein, which would have been otherwise tubularly retained. That L- and D-isomers are equally effective in reducing renal retention supports the view that simple neutralization of negative charges of the luminal tubular cell surface by positively charged molecules hinders the reabsorption of protein molecules because there is no luminal carrier known for D-lysine in the mammalian tubule cells (7), which would take up the D-isomer to the intracellular compartment. The fact that D-lysine also was effective when administered p.o. was at first surprising because one might expect that there are no effective intestinal transporters for the resorption of D-amino acids. However, in the high concentrations used, passive diffusion along a tremendous concentration gradient may be possible. Additionally, there may be low amounts of less specific carriers that are also capable of transporting D-isomers, as has been described recently (37). Presumably, a combination of both mechanisms delivers sufficiently high serum levels to block the kidney uptake of fragments effectively.

The slightly higher relative effectiveness of a single p.o. dose of L-lysine compared to multiple repeated i.p. injections may be due to differences in each route of administration to maintain a constant serum level. The slightly increased serum clearance of Fab′ fragments that was observed under lysine challenge is presumably due to the known increase of the renal plasma flow induced by amino acid administration (38).

The physiological mechanism that regulates the reduction of kidney uptake for larger molecules, such as $F(ab)_2$ fragments, must remain speculative at this time. With its molecular weight of 100,000, $F(ab)_2$ is certainly too large to be filtered through the glomerular basement membrane (28). In lysine-treated mice, no intact $F(ab)_2$ was found in the urine but only substances of lower molecular weight, which is in contrast to the observations with Fab′. Presumably, the catabolism of $F(ab)_2$ takes place elsewhere [*e.g.*, the liver (10)], and the smaller metabolized products would be filtered and excreted via the kidneys. Although the precise mechanism needs to be investigated further, the fact that the dose to the kidneys could be reduced for $F(ab)_2$ fragments by 75% is of special interest for RAIT with radiometal-conjugated $F(ab)_2$ fragments (5).

Higher retention of indium than of yttrium was also observed by our group for other antibodies (27) and is resulting in an overestimation of the actual ^{90}Y dose, when calculated from the ^{111}In biodistribution (27)[5] In clinical studies, it has also been shown that, despite similar intravascular kinetics of ^{111}In- and ^{90}Y-labeled IgG, the two radionuclides differed in their tissue biodistribution (39). The reason for this difference must remain speculative at this point (perhaps a better intracellular retention of indium than of yttrium), but potential instability of the chelate seems highly unlikely because no enhanced bone accretion of yttrium could be observed.

A major concern that needs to be solved before undertaking clinical trials on the effect of kidney uptake reduction in patients undergoing RAID with antibody fragments is the pharmacology and toxicity of the compounds used for this purpose. There are contradictory opinions on the toxicity of amino acids given in high doses (40). This has been made especially difficult because the toxicity of L-lysine seems to be species dependent. Zager *et al.* (36) found that high amounts of L-lysine in rats can cause acute renal failure, whereas clinical studies by Abel *et al.* (41) suggested a protective effect for the renal function of patients with acute tubular necrosis when amino acid solutions were given i.v. The known genetic defect of familial hyperlysinemia is not associated with any known symptoms (42). Because the toxicity of lysine seems to be restricted to its L-isomer (7), D-lysine should be metabolically inert and applicable without endangering the metabolic balance between the different amino acids and their metabolites (7), especially because no transmembrane transporters capable of taking up D-lysine are known in humans. Indeed, the

[5] T. M. Behr, R. M. Sharkey, M. E. Juweid, R. Stein, R. M. Dunn, M. J. Mattes, L. S. Shih, G. L. Griffiths, and D. M. Goldenberg, manuscript in preparation.

MTD of D-lysine in mice is approximately 1.4-fold higher than the MTD of the L-isomer.[6]

The toxicity of polylysine is still poorly understood. However, it is used in cell culture as a cell adhesion-mediating agent (43), which may suggest that severe toxicity could occur. The efficacy of p.o. administered lysine is encouraging because it would preclude the need for a long i.v. infusion and would thus present a much more convenient method for clinical use.

Thus, it is likely that a relatively simple approach can be used to reduce renal retention of labeled antibody fragments or peptides. Such a method may provide greater opportunities for utilizing these agents in diagnostic as well as therapeutic applications. In fact, preliminary clinical results are promising (13–15, 30).

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OIPE IAP45
NOV 07 2005
PATENT & TRADEMARK OFFICE

United States Court of Appeals for the Federal Circuit

04-1069

PANDROL USA, LP and PANDROL LIMITED,

Plaintiffs-Appellees,

v.

AIRBOSS RAILWAY PRODUCTS, INC.,
AIRBOSS OF AMERICA CORP., ROBERT M. MAGNUSON,
and JOSE R. MEDIAVILLA,

Defendants-Appellants.

Allen I. Rubenstein, Gottlieb, Rackman & Reisman, P.C., of New York, New York, argued for plaintiffs-appellees. With him on the brief was Raymond B. Churchill, Jr.

Richard R. Johnson, Shook, Hardy & Bacon, L.L.P., of Kansas City, Missouri, argued for defendants-appellants.

Appealed from: United States District Court for the Western District of Missouri

Senior Judge Scott O. Wright

United States Court of Appeals for the Federal Circuit

04-1069

PANDROL USA, LP and PANDROL LIMITED,

Plaintiffs-Appellees,

v.

AIRBOSS RAILWAY PRODUCTS, INC.,
AIRBOSS OF AMERICA CORP., ROBERT M. MAGNUSON,
and JOSE R. MEDIAVILLA,

Defendants-Appellants.

DECIDED: September 19, 2005

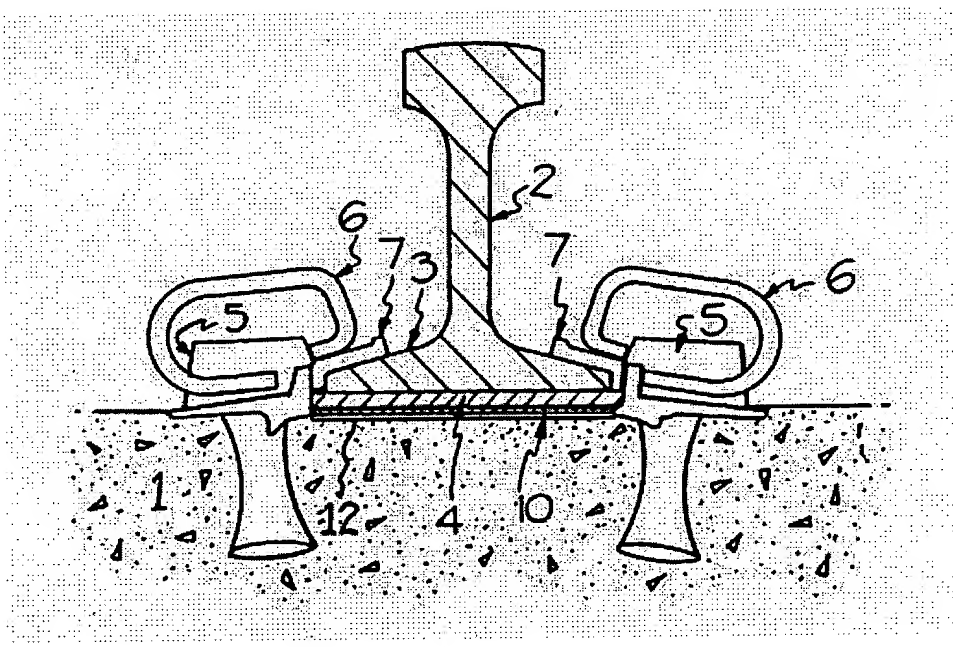
Before CLEVENGER, RADER, and DYK, Circuit Judges.

RADER, Circuit Judge.

The United States District Court for the Western District of Missouri granted summary judgment to Pandrol USA, LP and Pandrol Ltd. (Pandrol) because Airboss Railway Products, Inc., Airboss of America Corp., Robert M. Magnuson, and Jose R. Mediavilla (Airboss) did not produce clear and convincing evidence that U.S. Patent No. 5,110,046 (the '046 patent) is invalid. Pandrol USA, LP v. Airboss Ry. Prods., Inc., No. 99-0182-CV-W-SOW (W.D. Mo. Oct. 15, 2003) (Opinion). The district court determined that no reasonable juror could find that the '046 patent fails to satisfy the written description requirement of 35 U.S.C. § 112. Because the district court's ultimate determinations were correct, this court affirms.

I.

The '046 patent claims a railroad track fastening system. '046 patent, col. 2, ll. 45-66. Specifically, the patent claims a rail seat assembly that resists erosion of the concrete rail tie by interposing an abrasion-resistant plate and a layer of adhering material between the rail pad and the rail. Id. Figure 1 from the patent specification shows the invention:



The patent specification describes the preferred embodiment, in part, as follows:

> The improvement of this invention is to provide an abrasion plate 10 between the pad 4 and the tie 1. The plate 10 is smooth edged and incorporates recesses 11 to fit around the clamp supports or shoulder 5.
>
> The plate 10 may be bonded by layer 12 of adhesive (epoxy resin adhesives are preferred) to the tie 1 or an HDPE closed cell foam of 1.5 mm thickness of the same size and shape as plate 10 fitted between plate 10 and tie 1.

Id. at col. 2, ll. 33-41.

The district court determined on summary judgment that there was no genuine issue regarding whether the '046 patent's original specification satisfies the written description requirement of 35 U.S.C. § 112. Opinion at 7. Specifically, the district court declined to invalidate claims because the specification includes sufficient disclosure to support the claim limitations that include the terms "adhering material" and "sole means," id., both of which were added by amendment during prosecution. Those terms appear in claims 1 and 3. Claim 1 recites:

> An abrasion resistant rail seat for securing a rail to a concrete rail tie of the type in which the rail has a flange and is secured to a concrete rail tie by elastic rail clamps and an elastomeric rail pad insulates the rail from the rail tie, the improvement comprising interposing an abrasion resistant plate between said rail pad and said rail tie, said abrasion resistant plate forming a water tight seal with said rail tie, said abrasion resistant plate being wider than said rail and extending beyond the flange of said rail, and a layer of adhering material between said abrasion resistant rail plate and said rail tie for adhering said plate to said tie, said material being the sole means for adhering said plate to said tie so that the replacement of said abrasion resistant rail plate is facilitated.

'046 patent, col. 2, ll. 46-60 (emphases added). Claim 3 recites: "A rail seat as claimed in claim 2, wherein said adhering material is a closed cell foam pad of one to two millimetres [sic] in thickness and of similar shape to said plate." Id. at col. 2, ll. 63-66 (emphasis added).

This case has already been before this court twice. In the first appeal, this court affirmed the district court's grant of summary judgment of noninfringement of U.S. Patent No. 4,463,898 (the '898 patent), but vacated the grant of summary judgment of noninfringement of the '046 patent and remanded the case for further proceedings. Pandrol USA, LP v. Airboss Ry. Prods., Inc., 10 Fed. Appx. 837, 840-841 (Fed. Cir. Mar. 27, 2001) (Pandrol I). On remand, Pandrol prevailed, securing a judgment

awarding it damages and an injunction based on a finding of infringement of claim 3 of the '046 patent. Pandrol USA, LP v. Airboss Ry. Prods., Inc., No. 99-0182 (W.D. Mo. Mar. 28, 2002). In the second appeal, this court affirmed the determination of infringement, but vacated the district court's ultimate judgment awarding damages and an injunction pending resolution of the issue of invalidity. Pandrol USA, LP v. Airboss Ry. Prods., Inc., 320 F.3d 1354 (Fed. Cir. 2003) (Pandrol II). This appeal follows from that remand.

II.

This court reviews a district court's grant of summary judgment without deference. Kemco Sales Inc. v. Control Papers Co., 208 F.3d 1352, 1359 (Fed. Cir. 2000). Summary judgment is appropriate when the record shows that no issues of fact remain and that the moving party deserves judgment as a matter of law. See Fed. R. Civ. P. 56(c); Anderson v. Liberty Lobby, Inc., 477 U.S. 242, 248 (1986); Gen. Mills Inc. v. Hunt-Wesson, Inc., 103 F.2d 978, 980 (Fed. Cir. 1997). This court reviews compliance with the written description requirement as a question of fact. Purdue Pharma LP v. Faulding, Inc., 230 F.3d 1320, 1329 (Fed. Cir. 2000). This court reviews the issue of assignor estoppel under an abuse of discretion standard. Carroll Touch, Inc. v. Eletro Mech. Sys., Inc., 15 F.3d 1573, 1579 (Fed. Cir. 1993). Obviousness is a question of law premised on underlying findings of fact. Graham v. John Deere Co., 383 U.S. 1, 17-18 (1966).

Written Description

The district court observed that the "statements in the '046 patent are more than adequate to satisfy the written description requirement of 35 U.S.C. § 112." Opinion at

7. A patent specification must contain an adequate written description. 35 U.S.C. § 112, ¶ 1 (1994).

In 1967, this court's predecessor inaugurated use of § 112 to prevent the addition of new matter to claims. In re Ruschig, 379 F.2d 990 (CCPA 1967). Thus, in a recent application of the written description doctrine, this court noted: "The purpose of the written description requirement is to prevent an applicant from later asserting that he invented that which he did not; the applicant for a patent is therefore required 'to recount his invention in such detail that his future claims can be determined to be encompassed within his original creation.'" Amgen Inc. v. Hoechst Marion Roussel Inc., 314 F.3d 1313, 1330 (Fed. Cir. 2003) (citing Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1561 (Fed. Cir. 1991)).

Compliance with § 112 requires sufficient information in the specification to show that the inventor possessed the invention at the time of that original disclosure. See Vas-Cath, 935 F.2d at 1561 ("Adequate description of the invention guards against the inventor's overreaching by insisting that he recount his invention in such detail that his future claims can be determined to be encompassed within his original creation."). The possession test requires assessment from the viewpoint of one of skill in the art. Id. at 1563-64 ("[T]he applicant must . . . convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention." (emphasis in original)); Union Oil Co. of Cal. v. Atl. Richfield Co., 208 F.3d 989, 997 (Fed. Cir. 2000) ("The written description requirement does not require the applicant 'to describe exactly the subject matter claimed, [instead] the description must clearly allow

persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed.'" (citation omitted)).

With respect to the "adhering material" claim limitation, the original written description of the '046 patent describes a closed cell foam pad. During prosecution, amendments were made to both the claims and the specification. Thus, for the purposes of the written description analysis, this court's focus is on the original disclosure. In its Pandrol I claim construction, this court determined that "a closed cell foam pad is an 'adhering material.'" 10 Fed. Appx. at 842. In Pandrol II, this court restated: "Both the abstract and the preferred embodiment make clear that a gasket between the rail plate and rail tie, in and of itself, constitutes an 'adhering material.'" 320 F.3d at 1363. In its summary judgment determination, the district court properly reached the same conclusion. Opinion at 7. The written description of the '046 patent discloses an "adhering material," as claimed, in sufficient detail to show possession of the full scope of the invention.

The specification discloses that the invention provides an effective adhesive seal between the plate and concrete rail tie. '046 patent, col. 2, ll. 6-10. The original specification states:

> Preferably the abrasion plate may be adhered to the surface of the concrete tie to ensure that ingress of abrasive particles and water onto the surface of the rail tie is avoided.

Id. at col. 2, ll. 7-10. At another point, the specification describes an effective seal as "essential." Id. at col. 2, l. 6. The '046 patent then discloses two alternatives for achieving this feature: either with epoxy or with an HDPE closed cell foam gasket. The original abstract states:

> The plate may be bonded to the rail tie or a resilient gasket can be interposed between the rail tie and the plate.

'046 patent, abstract. The original specification also states:

> The plate 10 may be bonded by adhesive (epoxy resin adhesives are preferred) to the tie 1 or an HDPE closed cell foam of 1.5 mm thickness of the same size and shape as plate 10 is fitted between plate 10 and tie 1.

Specification of the '046 patent at 4 (as originally filed). Thus, these passages show that one way of providing an effective adhesive seal between the plate and concrete rail tie is a closed cell foam pad.

With respect to the "sole means" limitation, claim 1 says that the adhering material is the "sole means" for adhering the abrasion resistant plate to the rail ties. '046 patent, col. 2, ll. 57-58. The '046 patent describes and shows "a rail 2 which . . . sits on a rail pad 4 interposed between rail 2 and the concrete tie 1." Id. at col. 2, ll. 26-28. "The rail is held in place by rail clamp 6 which is held in clamp support 5" and has a toe portion that "bears down on rail flange 3 through the insulator 7." Id. at col. 2, ll. 29-32. Those passages, however, do not establish that the mechanical clamping system also performs the adhering function. The patent shows that the clamping system secures or clamps some parts mechanically but does not adhere. See id. at col. 1, ll. 66-67 ("However, once in this position [the plate] should not move significantly due to the clamping force of the rail clips."). The record does not show that the pressure exerted by the railed clips causes or contributes to the adhering performed by the HDPE foam. Thus, the disclosed adherents remain the "sole means" for that function.

In addition, the sole means limitation refers to the specific bonding of the rail tie to the rail pad to prevent erosion of the concrete rail tie with a watertight seal. Thus, the foam gasket primarily prevents erosion. In contrast, the primary purpose of the clamps

is to lock or hold the system in place. The district court correctly discerned that the specification provides adequate distinctions between clamping and adhering to show possession of the "sole means" aspect of the claimed invention.

Inventor's Testimony

Invoking the doctrine of assignor estoppel, the district court excluded Mr. Young's testimony. "Assignor estoppel is an equitable doctrine that prevents one who has assigned the rights to a patent (or patent application) from later contending that what was assigned is a nullity." Diamond Scientific Co. v. Ambico, Inc., 848 F.2d 1220, 1224 (Fed. Cir. 1988). Thus, an assignor and parties in privity with the assignor are estopped or barred from asserting invalidity defenses. Id. In this case, the district court invoked that doctrine to bar an assignor from testifying against the validity of its own patent.

Courts frequently mention four justifications for the doctrine of assignor estoppel: "(1) to prevent unfairness and injustice; (2) to prevent one [from] benefiting from his own wrong; (3) [to adopt the] analogy [of]. . . estoppel by deed in real estate; and (4) [to adopt the] analogy to a landlord-tenant relationship." Id. (quoting Hal Cooper, Estoppel to Challenge Patent Validity: The Case of Private Good Faith vs. Public Policy, 18 Case W. Res. 1122, 1128 (1967)).

This case relies primarily on the "unfairness and injustice" justification. "[A]n assignor should not be permitted to sell something and later assert that what was sold is worthless, all to the detriment of the assignee." Diamond, 848 F.2d at 1224. "The principle of fair dealing as between assignor and assignee of a patent whereby the assignor will not be allowed to say that what he has sold as a patent was not a patent has been part of the fabric of our law throughout the life of this nation." Id. (quoting

Scott Paper Co. v. Marcalus Mfg. Co., 326 U.S. 249, 260 (Frankfurter, J., dissenting)). Thus, assignor estoppel prevents an assignor from asserting that its own patent, for which it may have received value upon assignment, is invalid and worthless. The district court properly excluded Mr. Young's testimony on this basis.

The district court also correctly excluded Mr. Young's testimony because Airboss did not produce his expert report before trial. Under Federal Rule of Civil Procedure (FRCP) 26(a)(2), testimony offered as expert opinion requires the offer of an expert report prior to trial. Mr. Young's declaration contains expert opinion because he opines on claim construction and interpretation of the original application. Thus, without an expert report proffered for the record at any time, the district court correctly excluded this testimony on the basis of FRCP 26(a)(2).

Obviousness

The district court excluded some prior art allegedly relevant to the validity of the '046 patent under 35 U.S.C. § 103 because Airboss did not disclose it until the filing of a cross-motion for summary judgment. Opinion at 6. The district court noted that Airboss did not disclose this prior art when other prior art came forth in advance of trial. Id. In addition, Airboss did not propose any jury instructions on obviousness. Rather, the record shows that Airboss explicitly opposed any jury instructions on that subject. Id. Finally, Airboss informed the district court that it was not asserting invalidity based on obviousness. Id. Airboss asserts that the district court abused its discretion in refusing to entertain Airboss's obviousness challenge. The factors cited by the district court support its conclusion that Airboss had waived invalidity based on obviousness. The district court acted within its discretion.

III.

In sum, this court affirms the district court's decision that the '046 patent's specification satisfies the written description requirement of § 112. In addition, this court affirms the district court's decision to exclude Mr. Young's testimony in light of the doctrine of assignor estoppel and because Airboss did not produce a timely expert report under FRCP 26(a)(2). Finally, the district court did not abuse its discretion in refusing to entertain Airboss's obviousness challenge.

COSTS

Each party shall bear its own costs.

AFFIRMED

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/200,791	11/30/1998	THOMAS M. BEHR	018734/0161	9799

26633 7590 10/21/2004

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EXAMINER	
HELMS, LARRY RONALD	
ART UNIT	**PAPER NUMBER**
1642	

DATE MAILED: 10/21/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No. 09/200,791	Applicant(s) BEHR ET AL.	
	Examiner Larry R. Helms	Art Unit 1642	

OIPE IAP45 NOV 07 2005 PATENT & TRADEMARK OFFICE

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on 23 August 2004.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-9,11-21,23-29 and 31-41 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-9,11-21,23-29 and 31-41 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 6/30/04 has been entered.

2. It is noted that the response filed 8/23/04 states that claims 1-9, 11-21, 23-29, 31-37, 40-42 as presented here are in the form previously indicated as allowable in the Notice of Allowability mailed December 22, 2000. In response to this, upon further consideration the following Office Action is being sent out.

3. Claims 1-9, 11-21, 23-29, 31-41 are pending.

Claims 1, 2, 18-19 have been amended.

Claims 38-41 have been added.

Claims 1-9, 11-21, 23-29, 31-41 are under examination.

4. The text of those sections of Title 35 U.S.C. code not included in this office action can be found in a prior Office Action.

5. This Office Action contains NEW GROUNDS of rejections.

Claim Objection

6. Claim 38 is objected to because the term "polylysine" should be "poly-lysine" as recited in other claims.

Appropriate correction is required.

Priority

7. The instant application is a CIP of 08/407899 filed 3/21/95 (now US Patent 5,843,894). Claims 1 and 18 in the instant application recite the limitation of a method of reducing kidney retention of a protein conjugate. This limitation is not seen in the 08/407899 application. The 08/407899 application is directed to reducing renal uptake of antibody and antibody fragment conjugates which is a species of the now claimed genus of protein conjugates. The species of antibodies does not support the genus of just any protein conjugate. In addition, the glycoprotein conjugates and lipoprotein conjugates do not have support in the 08/407899 application (see claim 2 in the instant application). As such the claims are granted the priority date of the instant application, 11/30/98.

Rejections Withdrawn

8. The rejection of claims 1-21 and 23-37 under 35 U.S.C. 103(a) as being unpatentable over Behr et al (Cancer Research 55:3825-3834, 1995), and further in

view of Grey et al (U. S. Patent 5,380,513, issued 1/10/95, IDS #4) and Raines et al (U.S. Patent 5,840,296, filed 10/15/97) is withdrawn in view of the new grounds of rejections set forth below.

The following are NEW GROUNDS of rejections

9. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claim 38 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a NEW MATTER rejection.

Claim 38 has been added (not entered in the response filed 2/14/03 but entered now upon filing the RCE) and recites the limitation of a method comprising administering to a patient a "cytotoxic agent or imaging isotope" and additionally administering D-lysine or poly-lysine. The response filed 2/14/03 stated that the generic phrase in new claim 38 is the exact language contained in the '899 application and one skill in the art reading the specification as a whole would readily understand that applicants possessed a generic scope extending to all such cytotoxic or imaging agents that are susceptible to renal uptake (see page 3 of response). The response has been carefully considered but is deemed not to be persuasive. The instant specification is

directed to protein conjugates comprising cytotoxic or imaging agents and '899 specification is directed to antibody or antibody fragment conjugates comprising cytotoxic or imaging agents. There is no support in either application for methods using the cytotoxic or imaging agents alone that are not conjugates of protein or antibodies in a method with administration of D-lysine or poly-lysine. Applicant is required to provide specific support for the limitation in the application as originally filed of remove the limitation from the claim.

11. Claims 19 and 38 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of reducing kidney retention of a protein conjugate of a cytotoxic or imaging agent and administering poly-lysine or D-lysine having a molecular weight of 1-60kD, does not reasonably provide enablement for a method of reducing kidney retention of only a cytotoxic or imaging agent or just any metabolic product or peptide, polypeptides, glycoproteins, lipoproteins, antibodies or antibody fragments and administering poly-lysine or D-lysine having a molecular weight of 1-60kD. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in Ex parte Forman, 230 USPQ 546 (BPAI 1986). They include the nature of the invention, the state of the prior art, the relative skill of those in the art, the amount of direction or guidance disclosed in the specification, the presence

or absence of working examples, the predictability or unpredictability of the art, the breadth of the claims, and the quantity of experimentation which would be required in order to practice the invention as claimed.

The claims are broadly drawn to a method of reducing kidney retention with a protein conjugate that is a metabolic product of a peptide, polypeptide, glycoprotein, lipoprotein, antibodies or antibody fragments or a cancer therapeutic or diagnostic method of administering a cytotoxic agent or imaging agent and D-lysine or poly-lysine to reduce kidney retention of the agent. The specification discloses reducing the renal uptake of protein conjugates, in particular antibody conjugates by adding D-lysine, poly-lysine (see page 3). The specification does not enable the reduction of renal uptake of agents not conjugated to a protein or peptide or that just any metabolic products which are the protein conjugate that are reduced in renal uptake.

While the prior art does recognize reduction of renal uptake of protein conjugates as evidenced by Behr et al (Cancer 80:2591-2610, 1997) which teach reduction using antibody conjugates labeled with agents and addition of D-lysine (see entire document), there is no indication in the specification or the prior art to indicate that unconjugated agents would have reduced renal uptake by adding D-lysine or poly-lysine. In addition, Behr et al (supra) discloses that the kidney is the primary dose-limiting organ in RAIT with Fab fragments but does not provide any indication that unconjugated agents would be retained in the kidney and need D-lysine or poly-lysine for lowering uptake in the kidney. In addition, the prior art does not indicate that metabolic products or peptides,

polypeptide, glycoproteins, lipoproteins, or antibodies are reduced when added with D-lysine or poly-lysine in the kidney.

Therefore, in view of the lack of guidance in the specification and in view of the discussion above one of skill in the art would be required to perform undue experimentation in order to practice the claimed invention.

12. Claims 19, 38, and 40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claim 19 is indefinite for reciting "metabolic products thereof" because the exact meaning of the phrase is not clear. Does the phrase mean the conjugate is metabolized or broken down or the metabolic product is used as a conjugate or the metabolic product can be processed peptides? In addition, it is unclear if the agent is the metabolized product or if the protein part is the metabolized product. It is unclear what the phrase means and it is impossible to determine the meets and bounds of the phrase and claim.

b. Claim 38 recites said "imaging agent" in claim 38 and this phrase lacks antecedent basis in the claim.

c. Claim 40 is indefinite for reciting the trademark "ONCONASE" because the meaning of the name may be changed to refer to other compounds during the life of the patent. Also the name is not designated as a trademark. Amending the claim to recite the common generic form of the trademark as supported by the specification as

originally filed, would obviate this part of the rejection. Merely capitalizing the term does not overcome the rejection.

Claim Rejections - 35 USC § 102

13. Claims 1-8, 11-19, 23-28, 31-39, 41 are rejected under 35 U.S.C. 102(b) as being anticipated by Behr et al (Cancer Research 55:3825-3834, 1995).

The claims recite a method of reducing kidney retention of a protein conjugate or in a patient undergoing treatment comprising administering D-lysine or poly-lysine of 15-30kD or a combination of two compounds and a protein conjugate to a patient and the protein conjugate is not greater than 60 kD, wherein the conjugate is a imaging isotope or a therapeutic isotope, wherein the solution is administered to the patient as a continuous infusion, i.v., i.p, orally, one injection or a continuous infusion, wherein the conjugate is a radiolabeled hapten conjugate. This rejection is made because the application is not grated the priority of the '899 application due to no support for the genus of proteins as indicated above and because an antibody is a protein. In addition, claim 38 is granted the priority of the instant application because of the new matter rejection and the art is being applied to what is enabled which is a protein conjugate comprising a cytotoxic or imaging agent (see above).

Behr et al teach a method of reduction of renal uptake of a protein conjugate of an antibody fragment of Fab' of which when conjugated is less than 60kD comprising an imaging or therapeutic moiety in a patient (mouse model) with addition of D-lysine and poly-lysine (15-30 kD) and the solutions were administered by iv or ip or orally and two

compounds were administered together (see entire document, especially abstract, page 3826, 3827, 3rd paragraph, 3830, left column first paragraph).

Claim Rejections - 35 USC § 103

14. Claims 1-9, 11-21, 23-29, 31-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Behr et al (Cancer Research 55:3825-3834, 1995), and further in view of Grey et al (U. S. Patent 5,380,513, issued 1/10/95, IDS #4) and Raines et al (U.S. Patent 5,840,296, filed 10/15/97).

Claims 1-8, 11-19, 23-28, 31-39, 41 have been described supra. Claims 9, 20-21, 29, 40, recite wherein the compound is poly-D-lysine, wherein the targeting protein conjugate is ONCONASE. This rejection is made because the application is not grated the priority of the '899 application due to no support for the genus of proteins as indicated above and because an antibody is a protein. In addition, claim 38 is granted the priority of the instant application because of the new matter rejection and the art is being applied to what is enabled which is a protein conjugate comprising a cytotoxic or imaging agent (see above).

Behr et al teach a method of reduction of renal uptake of a protein conjugate of an antibody fragment of Fab' of which when conjugated is less than 60kD comprising an imaging or therapeutic moiety in a patient (mouse model) with addition of D-lysine and poly-lysine (15-30 kD) and the solutions were administered by iv or ip or orally and two compounds were administered together (see entire document, especially abstract, page 3826, 3827, 3rd paragraph, 3830, left column first paragraph). Behr et al does not

teach a protein conjugate comprising a ribonuclease or ONCONASE. These deficiencies are made up for in the teachings of Grey et al and Raines et al.

Grey et al teach a method to reduce renal retention of protein conjugates with lysine (see abstract and column 3, lines 44 to column 4, lines 2). Grey et al teach the conjugates comprise imaging agents and therapeutic agents (see column 7), that comprise cytotoxins and the proteins comprise receptors and enzymes as well as other proteins (see columns 5-6) Grey et al also teach administration orally, iv, ip, or the like (column 6, lines 1-5).

Raines et al teach conjugates comprising ribonuclease which have been effective in tumor patients (see column 1) and the decrease in renal function of Onconase may be the consequence of an inability to effectively clear the Onconase protein from the kidneys (see column 2, lines 52-57). Onconase is a 104 amino acid protein (see column 2, lines 34-35) which is not greater than 60 kD.

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced a method for reducing kidney retention of protein conjugates in a patient with administration of compounds of lysine or poly-lysine in view of Behr et al, Grey et al, and Raines et al.

One of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced a method for reducing kidney retention of protein conjugates in a patient with administration of compounds of lysine or poly-lysine in view of Behr et al, Grey et al, and Raines et al because Behr et al teach that kidney retention was reduced in conjugates by addition of lysine and poly-lysine

and that poly-lysine (15-30 kD) was more effective in reducing renal uptake and D-lysine should be metabolically inert (see page 3829 and 3832). In addition, one of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced a method for reducing kidney retention of protein conjugates in a patient with administration of compounds of lysine or poly-lysine in view of Behr et al, Grey et al, and Raines et al because Grey et al teach that protein conjugates comprising enzymes and added lysine can reduce renal uptake of the conjugates. In addition, one of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to have produced a method for reducing kidney retention of protein conjugates in a patient with administration of compounds of lysine or poly-lysine in view of Behr et al, Grey et al, and Raines et al because Raines et al teach "A cytotoxic ribonuclease that is readily cleared from the kidneys would be less likely to cause renal toxicity" (see column 2, lines 58-62). Thus it would have been obvious to one of ordinary skill in the art to produce a method of reducing renal uptake of protein conjugates that comprise ONCONASE conjugates in view of the teachings of Behr et al, Grey et al, and Raines et al.

Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Double Patenting

15. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11

F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

16. Claims 1-9, 11-21, 23-29, 31-41 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 38-47 of copending Application No. 10/438,219. Although the conflicting claims are not identical, they are not patentably distinct from each other. The claims in the instant application encompass and anticipate the claims in the 10/438,219 application. Both claim sets are directed to methods of reducing kidney retention of a protein conjugate or agents by administering D-lysine or poly-lysine in the range of 1-60kD.

This is a <u>provisional</u> obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

17. No claim is allowed.

18. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Larry R. Helms, Ph.D, whose telephone number is (571) 272-0832. The examiner can normally be reached on Monday through Friday from 6:30

am to 4:00 pm, with alternate Fridays off. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffery Siew, can be reached at (571) 272-0787.

19. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The Fax Center telephone number is 703-872-9306.

Larry R. Helms
571-272-0832

LARRY R. HELMS, PH.D
PRIMARY EXAMINER

Notice of References Cited

Application/Control No.	09/200,791
Applicant(s)/Patent Under Reexamination	BEHR ET AL.
Examiner	Larry R. Helms
Art Unit	1642

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U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
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	D	US-			
	E	US-			
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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Behr et al (Cancer 80:2591-2610, 1997
	V	
	W	
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*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
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